



**HAL**  
open science

## Ganglioside GM3-based anticancer vaccines: Reviewing the mechanism and current strategies

Jiaxu Zhang, Marco Terreni, Fang Liu, Matthieu Sollogoub, Yongmin Zhang

► **To cite this version:**

Jiaxu Zhang, Marco Terreni, Fang Liu, Matthieu Sollogoub, Yongmin Zhang. Ganglioside GM3-based anticancer vaccines: Reviewing the mechanism and current strategies. *Biomedicine and Pharmacotherapy*, 2024, 176, pp.116824. 10.1016/j.biopha.2024.116824 . hal-04601444

**HAL Id: hal-04601444**

**<https://hal.science/hal-04601444>**

Submitted on 5 Jun 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Ganglioside GM3-Based Anticancer Vaccines: Reviewing the Mechanism and**  
2 **Current Strategies**

3 (Running title: Review of GM3-Based Anticancer Vaccines)

4 **Authors and Institutes:**

5 Jiayu Zhang<sup>1,3</sup>, Marco Terreni<sup>2</sup>, Fang Liu<sup>1</sup>, Matthieu Sollogoub<sup>1</sup>, and Yongmin Zhang<sup>1,3\*</sup>

6 <sup>1</sup>Sorbonne Université, CNRS, Institut Parisien de Chimie Moléculaire, UMR 8232, 4 Place  
7 Jussieu, 75005 Paris, France

8 <sup>2</sup>Drug Sciences Department, University of Pavia, Viale Taramelli 12, 27100 Pavia, Italy

9 <sup>3</sup>College of Life Sciences, Northwest University, Xi'an 710069, China

10 **\*Correspondence to:**

11 Sorbonne Université, CNRS, Institut Parisien de Chimie Moléculaire, UMR 8232, 4 Place  
12 Jussieu, 75005 Paris, France; Tel: 33-144276153; Fax: 33-144275504; E-mail:  
13 yongmin.zhang@upmc.fr

1 **Abstract**

2 Ganglioside GM3 is one of the most common membrane-bound glycosphingolipids. The  
3 over-expression of GM3 on tumor cells makes it defined as a tumor-associated carbohydrate  
4 antigen (TACA). The specific expression property in cancers, especially in melanoma, make it  
5 become an important target to develop anticancer vaccines or immunotherapies. However, in  
6 the manner akin to most TACAs, GM3 is an autoantigen facing with problems of low  
7 immunogenicity and easily inducing immunotolerance, which means itself only cannot elicit a  
8 powerful enough immune response to prevent or treat cancer. With a comparative  
9 understanding of the mechanisms that how immune system responses to the carbohydrate  
10 vaccines, this review summarizes the studies on the recent efforts to development GM3-based  
11 anticancer vaccines.

12  
13 **Keywords:** GM3, TACA, Anticancer Vaccine, Glycoconjugate, Glycoimmunology

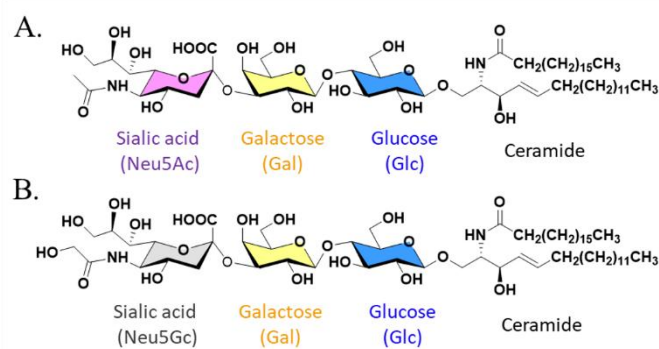
14  
15 **Abbreviations:**

16 **APC**, antigen-presenting cell; **BCR**, B cell receptor; **BSA**, bovine serum albumin; **CDC**,  
17 complement dependent cytotoxicity; **CMAH**, cytidine mono-phospho-*N*-acetylneuraminic  
18 acid hydroxylase; **COSMC**, core1-specific molecular chaperone; **DC**, dendritic cell; **ELISA**,  
19 enzyme-linked immunosorbent assay; **TACA**, tumor-associated carbohydrate antigen; **GSL**,  
20 glycosphingolipid; **HSA**, human serum albumin; **iNKT**, invariant natural killer T; **KLH**,  
21 keyhole limpet hemocyanin; **LacCer**, lactosylceramide; **LPS**, lipopolysaccharide; **mAb**,  
22 monoclonal antibody; **MHC**, major histocompatibility complex; **MPLA**, mono-phosphoryl  
23 lipid A; **OVA**, ovalbumin; **PAMP**, pathogen-associated molecular pattern; **PRR**, pattern  
24 recognition receptor; **TCR**, T cell receptor; **TME**, tumor microenvironment; **TTox**, tetanus  
25 toxoid; **TLRs**, toll-like receptors; **VSSP**, very small size proteoliposomes; **VLP**, virus-like  
26 particle;

27 For monosaccharides: **Glc**, glucose; **Man**, mannose; **Gal**, galactose; **GlcNAc**,  
28 *N*-acetyl-glucosamine; **GalNAc**, *N*-acetyl-galactosamine; **Fuc**, fucose; **Neu5Ac**,  
29 *N*-acetylneuraminic acid; **Neu5Gc**, *N*-glycolylneuraminic acid

## 1 1. Introduction

2 Ganglioside GM3 is one of the most common membrane-bound glycosphingolipids (GSLs)  
3 in tissues, which is composed of a sialic acid-containing tri-saccharide glycan attached to a  
4 lipophilic ceramide (Figure 1A). This structure allows the GM3 to be anchored into the  
5 phospholipid bilayer of eukaryotic cells with the ceramide moiety, leaving the glycan exposed  
6 on the cell surface and performing a series of complex biological and physiological functions.  
7 For example, GM3 rafting on cell membrane could form complexes with various co-localized  
8 structures (e.g. growth factors, insulin receptors and tyrosine kinases) by non-covalent  
9 (hydrophobic, electrostatic) bonds [1]. As a result, these complexes dynamically interact with  
10 the glycolipid-enriched microdomains to regulate downstream switches, thereby regulating  
11 the cellular signal transduction, cell proliferation, membrane transportation, and cell adhesion,  
12 etc [2-4]. In addition to GM3 that exists in a lipid raft model on the cell surface, there is also a  
13 small amount of free GM3 in the sera (6  $\mu\text{g}/\text{mL}$ ), and this content remains stable unless  
14 metabolic-related diseases such as type 2 diabetes and hyperlipidemia causes a significant  
15 increase (1.5-fold) in serum GM3 [5].



16 **Figure 1.** Representative structures of ganglioside GM3. **A.** Neu5Ac-GM3; **B.** Neu5Gc-GM3

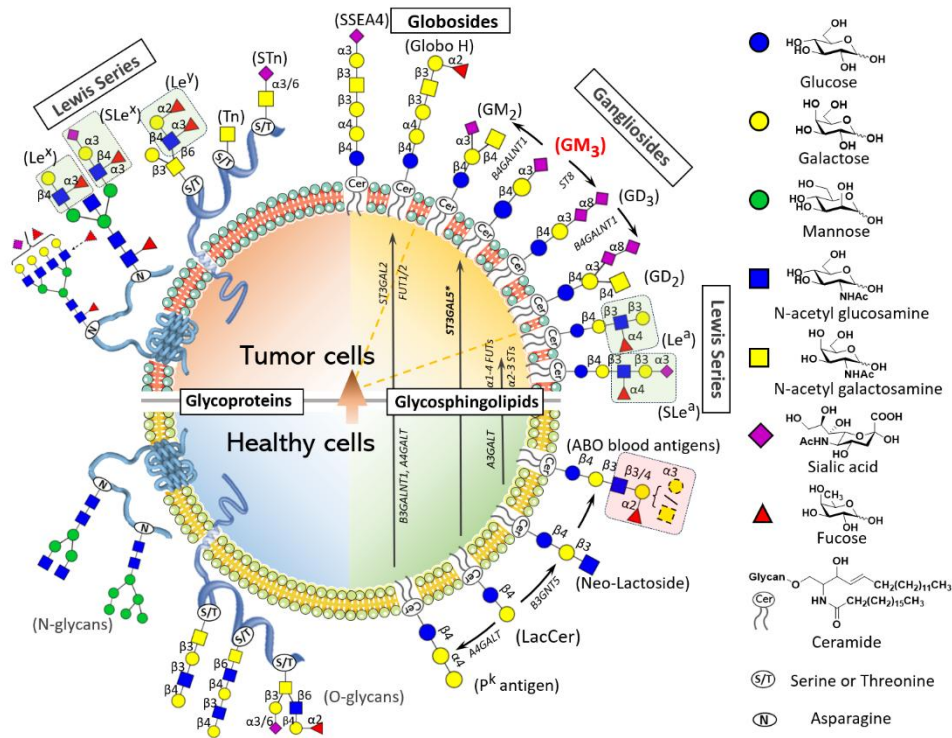
17 Ganglioside GM3 is over-expressed on some tumor cells, which makes it defined as a  
18 tumor-associated carbohydrate antigen (TACA). Studies have already identified that GM3 is  
19 significantly overexpressed in melanoma and not found in healthy skin or skin neighboring  
20 primary melanoma [6, 7], and the content of GM3 on cancer cells is positively correlated with  
21 the metastasis ability [8, 9]. It is worth mentioning that an important GM3 variant,  
22 Neu5Gc-GM3 (Figure 1B), was detected in melanoma, colon and breast cancer cells and was  
23 not present in other human healthy somatic cells [10, 11].

1 Since TACAs can play a role of antigenic epitopes and then trigger downstream immune  
2 responses, immunotherapy research that aims to target GM3 has always been a research  
3 hotspot. However, a major drawback of GM3 anticancer vaccines is the poor immunogenicity  
4 (ability to induce immune response) and antigenicity (ability to bind immunoglobulins), due  
5 to the autoantigen property which leads to immune tolerance and immune evasion [12, 13]. In  
6 particular, the tumor microenvironment (TME) caused by the increase of sialylation during  
7 tumor development will act like a "cloak" to protect tumor cells and reduce immune responses  
8 [14]. With a comparative understanding of the mechanisms that how immune system  
9 responses to the carbohydrate vaccines, this review summarizes the studies on the  
10 development of GM3 based anticancer vaccines in recent years.

## 11 **2. Glycosylation and tumor-associated carbohydrate antigens (TACAs)**

12 Glycosylation is an important post-translational pathway which enzymatically attaches  
13 oligosaccharides to proteins and lipids to form glycoproteins and GSLs [15, 16]. Major  
14 classification of glycoproteins in eukaryotic cells can be classified into *N*-glycosylated and  
15 *O*-glycosylated glycoproteins [17]. As shown in Figure 2, the *N*-glycans sharing a  
16 penta-saccharide core structure ((Man)<sub>3</sub>- (GlcNAc)<sub>2</sub>-), while the most common  
17 *O*-glycosylation is starting from *N*-acetyl-galactosamine (GalNAc). GSLs consist of a  
18 hydrophobic ceramide (Cer) moiety anchoring the outer layer of cell membranes and a glycan  
19 moiety extending into the extracellular matrix due to the hydrophilicity [18]. Generally, the  
20 glycoside moiety of GSLs starts from glucose, and various monosaccharides extend and  
21 branch the glycan structure under the action of glycosyltransferases to form diverse complex  
22 oligosaccharides (e.g. p<sup>k</sup> series, Neo-Lactosides, Globosides and Gangliosides) [19]. The most  
23 representative human functional GSLs are the ABO blood group antigen system. These  
24 carbohydrate antigens, present on red blood cells, differ by a monosaccharide residue at the  
25 terminal end of the glycan, yet they impose significant limitations on blood transfusion  
26 compatibility [20]. There is new evidence that mammals use RNA as a third scaffold for  
27 glycosylation. Those so-called "glycoRNAs" which consisted of small noncoding RNAs and  
28 sialylated glycans were present in multiple cell types and can interact with anti-dsRNA  
29 antibodies and members of Siglec receptors [21]. This discovery will greatly promote

1 research in the fields of glycoscience and help scholars more clearly understand the important



2 role of glycans in life activities.

3 **Figure 2.** Overview of the representative human cell glycosylation and tumor associated carbohydrate  
 4 antigens (TACAs). *N*-glycans possess a common structure of (Man)<sub>3</sub>(GlcNAc)<sub>2</sub> and are linked to the  
 5 asparagine side chains on membrane proteins; most *O*-glycans start with GalNAc and are linked to serine or  
 6 threonine residues on proteins; glycosphingolipids are anchored to the cell membrane via their hydrophobic  
 7 lipid tails. Under the action of glycosyltransferases and glycosidases (some examples marked on the black  
 8 arrows), a wide variety of glycans are elongated and branched, performing biological functions. During  
 9 cancer initiation and progression, abnormal glycosylation leads to the generation of various specific TACA  
 10 structures (e.g. SSEA4, Globo H, GM2, GM3, GD3, GD2 and Lewis Series. Of them, GM3 is highlighted in  
 11 red). To explain, there are only some representative glycans to illustrate the abnormal glycosylation. On  
 12 cancer cells, abnormal glycosylation manifests as an increased or decreased trend in glycan alterations, which  
 13 means the various structures presented here are not exclusively expressed in either normal or cancer cells.

14 During the development of cancer, aberrant glycosylation may occur in both glycoproteins  
 15 and glycolipids. For the *N*-glycans, based on the common penta-saccharide core structure, the  
 16 tumor-related aberrant glycosylation often manifests as multi-antennary galactose branching  
 17 [22], bisecting GlcNAc branching [23], fucosylation and sialylation [24, 25]. The *O*-glycans  
 18 normally form as extending and branching oligosaccharides beginning with GalNAc.  
 19 However, in cancers, because of the mutations in the core1-specific molecular chaperone  
 20 (COSMC) and competition of the sialyltransferase ST6GalNAc-I, it always shows an  
 21 over-expression of the Tn (“Tn” stands for truncated) antigens or sialyl Tn (STn) in cancer

1 related glycoproteins [26]. One of the most thoroughly studied breast cancer associated  
2 glycoprotein is mucin, which is a type I transmembrane glycoprotein with a unique  
3 extracellular domain consisting of lots of tandem repeat of 20 amino acids  
4 (HGVTSAPDTRPAPGSTAPPA) providing abundant Ser and Thr as potential sites for Tn or  
5 STn *O*-glycosylation [27].

6 As the most basic precursor of GSLs, lactosylceramide (LacCer) leads to the  
7 ganglioside-series pathway. GM3 is synthesized by the addition of a sialic acid to LacCer  
8 with a CMP-sialic acid donor catalyzed by the sialyltransferase ST3GAL5. Further extension  
9 by another sialyltransferase ST8 allows CMP-sialic acid to attach onto the position-8 of sialic  
10 acid and then form GD3 and other poly-sialylated gangliosides. Under the catalysis of  
11 glycotransferase B4GALNT1, GM3 and GD3 can extend the GalNAc branch to produce the  
12 corresponding ganglioside GM2 and GD2 [28]. In the similar way, other types of GSLs are  
13 extended into globoside TACAs such as SSEA4 and Globo H under the action of abnormally  
14 overexpressed glycosyltransferases in tumors, or produce special terminal structures such as  
15 the Lewis series. For more than twenty years, these TACAs mentioned above have  
16 demonstrated potential usefulness in strategies for cancer immunotherapy [29]. Some of them  
17 have already been well-developed: e.g. Racotumomab<sup>®</sup> (anti-Neu5Gc-GM3 monoclonal  
18 antibody (mAb) for non-small cell lung carcinoma), Theratope<sup>®</sup> (STn-keyhole limpet  
19 hemocyanin (KLH) glycoconjugate vaccine for breast cancer) and OPT-822 (Globo H-KLH  
20 glycoconjugate vaccine with adjuvant OPT-821 for breast cancer) have been achieved phase  
21 III clinical trials [30-32]. The approval of Dinutuximab<sup>®</sup> (a chimeric anti-GD2 mAb) for the  
22 treatment of high-risk neuroblastoma represents a significant achievement, marking the first  
23 endorsement by the U.S. Food and Drug Administration (FDA) for a novel agent targeting a  
24 TACA in 2015 [33].

25 In short, glycosylation is an extremely complex, delicate and systematic molecular  
26 regulation process. It gives tumors special cell epitopes, special signal transduction pathways  
27 and special TME. All of these provide unlimited possibilities for developing immunotherapy  
28 targeting TACAs, but also pose great challenges to researchers.

### 29 **3. Expression of ganglioside GM3 on cancer cells**

1       Ganglioside GM3 is widely present in virtually all types of animal cells and exhibits  
2 over-expression in various human cancers, including melanomas, lung, and brain cancers [34,  
3 35]. GM3 expression in melanomas has been extensively investigated. Reports indicate a  
4 significantly higher percentage of GM3 existence in both primary and metastatic melanomas  
5 compared to GD2 [36]. Additionally, there is a strong correlation between GM3 and GD3  
6 expressions in both primary and metastatic melanomas. Notably, GM3 is absent in normal  
7 skin and the skin adjacent to primary melanomas, in contrast to GD3. The onset of GM3  
8 expression occurs at an early stage of melanocyte differentiation, facilitating entry into the  
9 dermis of the skin [7, 37]. The detection of GM3 on the surface of tumor cells is a critical  
10 determinant of the metastatic phenotype [38]. Cell line B16 is derived from the classic  
11 melanoma mouse model C57BL/6, which is well established and widely used as tumor model  
12 to study melanoma metastases, melanoma-specific immune cells reactions and  
13 pharmaceutical activities [39]. Studies have indicated that GM3 density can influence  
14 antigenicity. The anti-GM3 mAb M2590 interacts with GM3 over-expressed melanoma B16  
15 cells while exhibiting no reactivity with corresponding normal tissue [40]. Therefore,  
16 researchers generally believe that the densely packed cluster formed by GM3 on the  
17 melanoma cell membranes is the key to the action of drugs or antibodies [41-43].

18       Considerable interest has been directed towards a GM3 variant, Neu5Gc-GM3, which is  
19 distinguished by the presence of *N*-glycolylneuraminic acid (Neu5Gc) rather than  
20 *N*-acetylneuraminic acid (Neu5Ac) [44, 45]. While Neu5Gc-GM3 is commonly synthesized  
21 in most mammals, normal human cells lack this variant due to an inactivating mutation in the  
22 human cytidine mono-phospho-*N*-acetylneuraminic acid hydroxylase (CMAH) gene [46].  
23 However, Neu5Gc-GM3 is frequently observed at elevated levels in human cancers, including  
24 melanoma, breast, colon and lung cancer [47, 48]. This phenomenon is attributed to the  
25 cancer cell's metabolic incorporation of Neu5Gc derived from dietary sources, particularly  
26 red meat [11]. The higher metabolic rate of Neu5Gc may result in the preference of  
27 expression by tumor cells [49]. In a recent investigation, it was proposed that the succinate  
28 dehydrogenase complex's subunit B, possessing a Fe<sub>2</sub>S<sub>2</sub> domain resembling the deleted gene  
29 fragment of the human CMAH protein, might interact with this protein, offering an alternative  
30 pathway for generating Neu5Gc during hypoxia [50]. This may offer an explanation why



1 metabolically active tumor cells exhibit such an abnormal expression of Neu5Gc-GM3.

2 Another GM3 associated molecule, GM3 lactone, was also found in human and mouse  
3 melanoma cells [43]. The carboxyl group on sialic acid undergoes condensation with the  
4 2-hydroxyl group of the adjacent galactose, forming a lactone. This formation is likely  
5 facilitated by the lower pH of TME within tumor cells and potentially influenced by a distinct  
6 conformation of GM3 ganglioside induced on the tumor cell surface due to its local high  
7 density [51]. The specificity of GM3 expression on tumor cells and the variant potential of the  
8 molecule itself make them promising for the development of anti-tumor immunotherapy [52].

#### 9 **4. Principles of glycoimmunology**

10 The development of vaccines against carbohydrates was first pursued to immunize against  
11 bacterial pathogens. The surface of bacteria contains abundant lipopolysaccharide (LPS)  
12 structures, of which the polysaccharides are generally rich in galactose and sialic acid [53, 54].  
13 Mammals have an innate immune system with conserved pattern recognition receptors (PRRs)  
14 that can recognize the certain glycopatterns from LPSs. For example, there are three major  
15 classes of carbohydrate-recognized lectins as pathogen-associated molecular patterns  
16 (PAMPs), galectins, Siglecs and C-type lectin receptors [55]. This is an early defense  
17 mechanism against bacterial infections. In addition to this early protection, immunizing the  
18 body with a certain dose of LPS antigen can activate a more specific humoral immune  
19 response, produce corresponding immunoglobulin antibodies, and trigger a downstream  
20 immune cascade. It is first time to report in 1917 that there was a specific soluble substance  
21 which could induce antibodies for *Pneumococci*, and it was subsequently identified as  
22 polysaccharide [56, 57]. Nowadays, vaccines based on bacterial LPSs have been well  
23 developed and routinely used in an immunization schedule to prevent people from  
24 *Haemophilus influenzae* type B, *Streptococcus pneumoniae* and *Neisseria meningitidis* [58,  
25 59].

26 The application of bacterial LPS vaccines has confirmed that the effectiveness of glycans  
27 or glycopatterns are immunogenic substances. Abnormal glycosylation and their  
28 corresponding product TACAs become hallmarks of cancer and can serve as a source of novel  
29 clinical biomarker developments, providing a set of specific targets for therapeutic

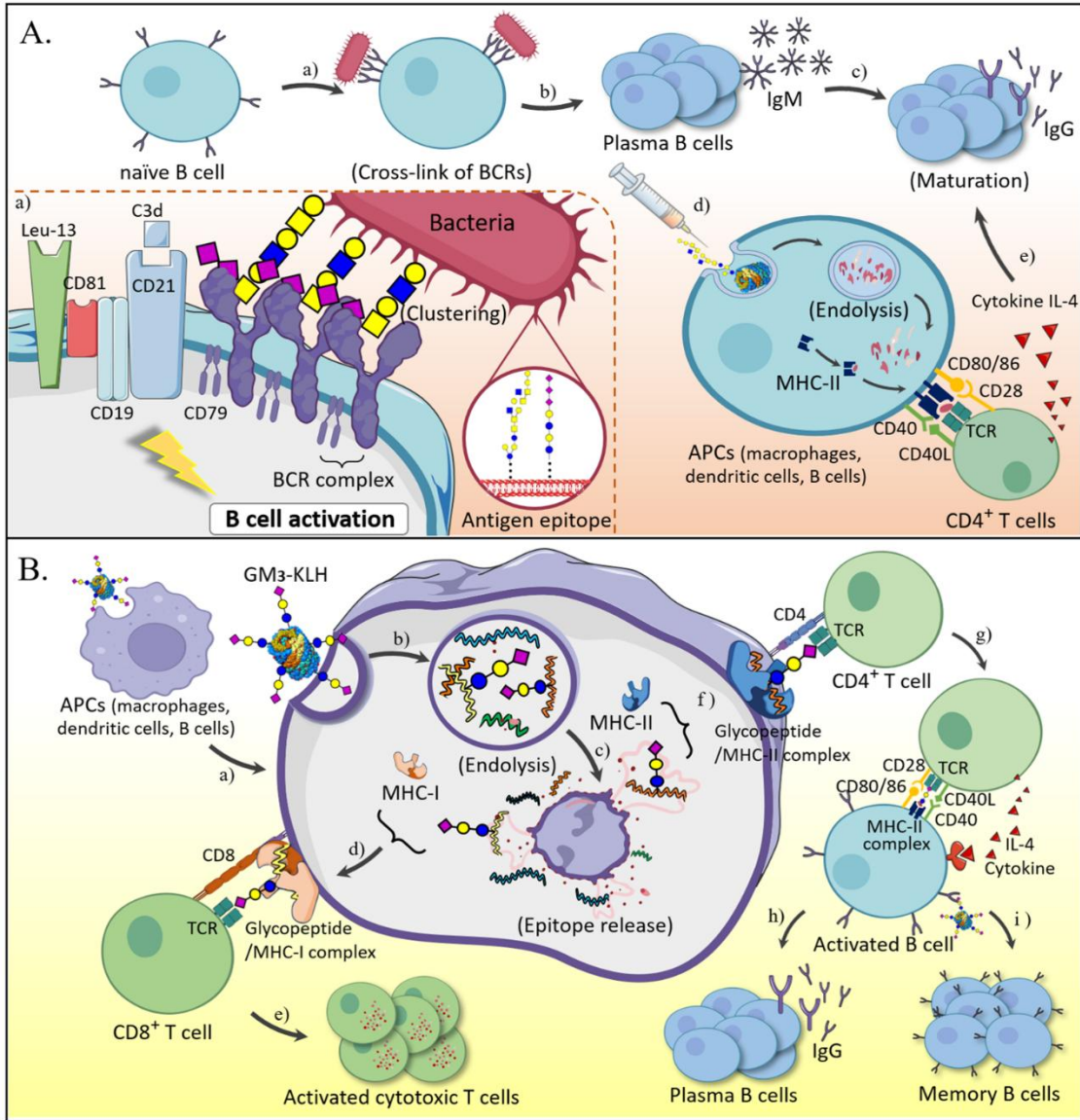
1 intervention. Over the past 30 years, the TACAs have demonstrated potential usefulness in  
2 strategies for cancer immunotherapy, including the production of vaccines, mAb therapy and  
3 the generation of CAR-T cells [60]. Of them, there are nine TACAs (GD2, GD3, GM3,  
4 Fucosyl-GM1, Globo H, poly-sialic acid, Tn, STn, SLe<sup>a</sup>) were explicitly included in the  
5 tumor-associated antigen list by the National Cancer Institute of the United States [61]. Here,  
6 the three major immune pathways of carbohydrate vaccines are compared.

#### 7 **4.1 Polysaccharide vaccines activate B cells by crosslink to the receptor clusters**

8 Polysaccharide vaccines contain numerous identical antigenic epitopes and can stimulate B  
9 cells through the cross-link interaction with the B cell receptors (BCRs) in a polyvalent  
10 fashion [62]. This kind of humoral immune process is usually associated with the  
11 development of antibacterial vaccines. In vaccinology it is widely used that inactivated  
12 pathogenic bacteria (such as Bacillus Calmette-Guérin) or their polysaccharides isolated by  
13 bacterial lysis or precisely synthesized by chemical methods [63, 64].

14 These polysaccharides will be firstly transported to the marginal zone of the spleen where  
15 contains abundant naive B cells and enriched with the complement receptor 2 (also known as  
16 CD21). The sketch of this pathway is shown in Figure 3A and described as follows: a)  
17 Bacterial polysaccharides generally consist of many repeating glycopattern units with an  
18 average size of 40-80 nm [65]. When the B cells recognize and bind to such polymeric  
19 immunogens, the BCRs (a complex consisting of immunoglobulin and CD79) spatially  
20 aggregate on the membrane. At this point, BCRs will cluster into groups (10-20 BCRs per  
21 cluster), inducing B cell activation and lowering the threshold for antigen affinity [66].  
22 Clustered BCRs, with the co-activation of complex of complement C3d and CD21, form a  
23 complex with CD19, CD81 and Leu-13, activating downstream protein tyrosine kinases to  
24 activate B cells [67]. b) Then, the activated B cells rapidly differentiate and proliferate into  
25 mature plasma B cells, producing IgM. The production of IgM is a rapid process, typically  
26 occurring within the first week of primary immunization. c) Upon successful initiation of the  
27 immune response to polysaccharides by this pathway, B cells require further maturation in the  
28 germinal center of the spleen or lymph nodes: transitioning from the IgM to the IgG isotype  
29 and differentiate to memory B cells which ensures a more effective and long-lasting humoral

1 immune protection [68]. Furthermore, due to the low or absent expression of CD21 on B cells  
 2 in children under 2 years of age, these polysaccharide vaccines often fail to confer early  
 3 immunity in newborns [69].



4 **Figure 3.** Schematic diagram of immune responses to vaccines with carbohydrate as antigen. **A.** T cell  
 5 independent immune pathway triggered by bacterial lipopolysaccharides (LPS), and an example using LPS-KLH  
 6 vaccine to demonstrate how glycoconjugates activate a T cell-dependent immune response: (a) To combine with  
 7 the LPS exposed on the bacterial surface, the BCRs are spatially aggregated. Then the B cell intracellular signaling  
 8 cascade is activated; (b) The activated B cells differentiate and proliferate into mature plasma B cells, producing  
 9 IgM; (c) B cells maturation and switch to IgG production; (d) Immunized with LPS-KLH conjugate vaccine, the  
 10 antigen presenting cells (APCs) uptake the glycoconjugates. Endolysis causes the decomposition of  
 11 glycoconjugates into peptides or glycopeptides, and the glycopeptides are captured by the type-2 major  
 12 histocompatibility complex (MHC-II), and transferred to the surface recruiting CD4<sup>+</sup> helper T cells. (e) Activated  
 13 helper T cells release cytokine IL-4 which further boost the maturation of B cells and induce the IgG production. **B.**  
 14 T cell dependent immune pathway triggered by immunogenic glycoconjugate (GM3-KLH as example): (a)

1 Glycoconjugate is endocytosed by APCs; (b) Endolysis causes the peptides and glycopeptides; (c) The epitopes are  
2 released and face different fates depending on peptide length; (d) Shorter peptide (8-10 residues) is captured by  
3 MHC-I and transferred to the surface recruiting CD8<sup>+</sup> T cells; (e) Recognizing the presented GM3 antigen with the  
4 T cell receptor (TCR), cytotoxic T cells are activated and matured; (f) Longer peptide (10-25 residues) is captured  
5 by MHC-II and transferred to the surface recruiting CD4<sup>+</sup> T cells; (g) The activated CD4<sup>+</sup> helper T cell recognize  
6 the B cell which endocytosed and presented the GM3 antigen. With activation of co-stimulatory molecules (CD28  
7 and CD40L) by the corresponding ligation, the helper T cell releases cytokines to boost the activation of B cell; (h)  
8 The activated B cells further differentiate to plasma B cells and produce IgG, (i) or differentiate to memory B cells.

9 Carbohydrates are *per se* T cell-independent type 2 antigens which means the carbohydrate  
10 itself only cannot activate the major histocompatibility complex (MHC) molecules involving  
11 cellular immune pathway. To break this limitation and take advantage of the helper T cells,  
12 glycoconjugate vaccines have been developed through the conjugation of polysaccharides to  
13 highly immunogenic carrier proteins. The concept of glycoconjugate vaccines was first  
14 developed by Avery and Goebel in 1931, of where they reported that the immunogenicity of  
15 bacterial polysaccharides could be enhanced by covalently linking them onto an appropriate  
16 carrier protein [70]. As illustrated in the supplementary pathway shown in Figure 3A: d)  
17 When immunization with glycoconjugates containing specific carbohydrate antigen epitope  
18 from those LPSs, the conjugates are internalized inside the antigen-presenting cells (APCs),  
19 e.g. B cells, macrophages and dendritic cells (DCs). The protein component of  
20 glycoconjugates is digested by proteases in the endosomal compartment, forming peptides  
21 and glycopeptides in a slightly acidic environment within the endosomes. Some suitable  
22 fragments will bind within the groove structure of MHC-II molecules, forming a  
23 peptide/glycopeptide-MHC-II complex. This complex will then be transported to the surface  
24 of B cells, recruiting CD4<sup>+</sup> T cells through CD40 and CD80/86. When the  $\alpha\beta$  T cell receptor  
25 recognizes the glycopeptide-MHC-II complex and co-stimulated by the ligand-binding of  
26 CD40L and CD28, the CD4<sup>+</sup> T cells release interleukin-4 (IL-4). e) Interaction with IL-4  
27 makes the B cells to induce IgM to IgG class switching (B cell maturation) that results in  
28 polysaccharide-specific high-affinity IgG antibody production [71]. This T cell dependent  
29 pathway also allows the differentiation of antigen-specific memory B cells and T cells and  
30 provides efficient prophylactic immunity [72].

#### 31 **4.2 Glycoconjugate vaccines activate the helper T cells by MHC presenting**

1 Given that glycans can be recognized by the immune system and immunoglobulins,  
2 targeting TACAs on tumor cells represents a promising avenue for the development of cancer  
3 immunotherapy. Compared to the bacterial polysaccharide antigens, TACAs have a lower  
4 degree of polymerization, consisting of oligosaccharides composed of 2~8 monosaccharide  
5 units [73, 74]. They cannot directly trigger the B cell immune pathway because the size of  
6 TACA molecules is not enough to cross-link to BCRs and make the BCRs come into cluster  
7 [66]. After it was proven that glycoconjugates with immunogenic carrier proteins can  
8 effectively activate the MHC dependent T cell pathway, making TACA molecules in form of  
9 glycoconjugates made it possible to develop carbohydrate based anti-tumor vaccines [75].

10 The strategy to utilize TACA based glycoconjugate vaccines is based on the classic  
11 adaptive immune system, also known as the acquired immune system, which relies on the  
12 ability to distinguish “self” antigens from pathogen-derived antigens [76]. Take the immune  
13 process of GM3-KLH glycoconjugate as an example, this pathway is illustrated as Figure 3B:

14 a) Upon conjugation an immunogenic carrier protein, the glycoconjugates will be firstly  
15 recognized as foreign substances and swallowed by APCs, including macrophages, DCs and  
16 B cells [77]. b) During this endocytosis process, the glycoconjugate molecules are surrounded  
17 by an area of cell membrane, which then buds off inside the APCs to form a vesicle  
18 containing the ingested contents. c) After internalization into APCs, the glycoconjugates are  
19 digested by proteases to release peptides, and glycopeptides bringing TACAs. d) Specific  
20 8-10 residue long peptides or glycopeptides are then transported to the lumen of endoplasmic  
21 reticulum for loading on MHC-I to form a complex [78]. In particular, there are two long  
22  $\alpha$ -helices (45 kDa of each) form two walls of a groove-shape like structure. The proper  
23 peptides could lie into the groove, and, in the case of glycopeptides, the glycans will extend  
24 out [79, 80]. The glycopeptide-MHC-I complex are then transferred onto the cell surface of  
25 APCs and recruit CD8<sup>+</sup> T cells. e) The T cell receptor (TCR) is a disulfide-linked  
26 membrane-anchored heterodimeric protein normally consisting of the highly variable  $\alpha$ - and  
27  $\beta$ -chains known as  $\alpha\beta$ -TCR (95% of all TCRs). The remaining 5% is  $\gamma\delta$ -TCR composed of  $\gamma$ -  
28 and  $\delta$ -chain [81]. Studies have shown that  $\gamma\delta$ -TCR is related to the recognition of the glycan  
29 moiety presented by the glycopeptide-MHC-I complex [82]. Specific TCR interacting with  
30 the antigen presented by MHC-I leads to activation and proliferation of the T cells, which

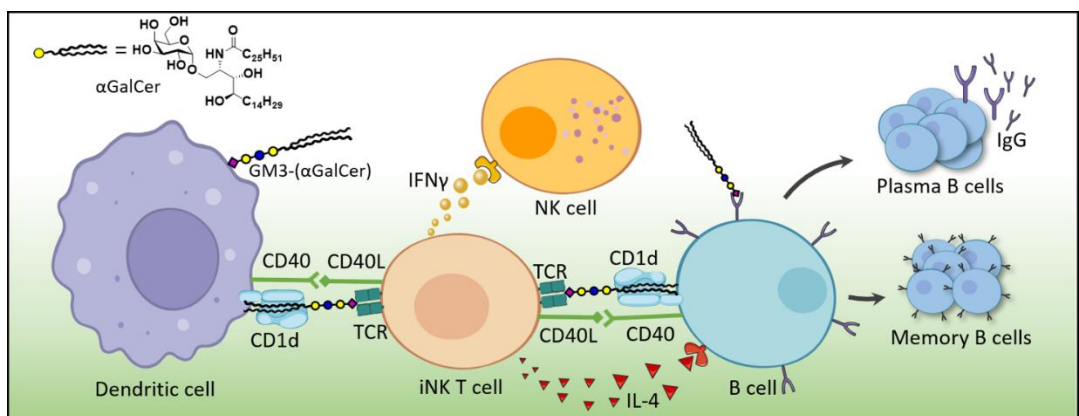


1 further differentiate to mature cytotoxic T cells [83]. These cytotoxic cells will act like the  
2 “monitors” to monitor tissues and cells according to the specific antigen information received,  
3 e.g. GM3 as example. If cancer cells expose the GM3, they will be eliminated by these  
4 cytotoxic T cell by downstream pathways.

5 On the other hand, these glycopeptide fragments also activate B cells and produce  
6 antibodies under the CD4<sup>+</sup> T cell assisted pathway: f) In this pathway, glycopeptides bind to  
7 MHC-II molecules to form a complex. Similarly, MHC-II consists of an  $\alpha$  (30-34 kDa) and a  
8  $\beta$  (26-29kDa) subunit to form a groove-like structure with openings at both ends which allows  
9 a longer peptide (10-25 residue long) could be accommodated [84, 85]. Export of the  
10 glycopeptide-MHC-II complex to the surface of APCs allows interactions with TCRs on  
11 CD4<sup>+</sup> T cells to initiate effector and regulatory functions. g) In particular, the activated CD4<sup>+</sup>  
12 T cells (also known as helper T cells) can make similar interaction with B cells. h) Thereby,  
13 the activated B cells are instructed by interleukins to mature and produce IgG antibodies. i)  
14 The memory B cells are also differentiated in this step.

### 15 4.3 Glycolipid vaccines activate the iNKT cells by CD1d presenting

16 In recent years, a strategy has emerged that uses glycolipid molecules as immune agonists  
17 to present oligosaccharide antigens and generate immune responses. The glycolipids are  
18 found to be immunomodulatory molecules which have the ability to activate a unique subset  
19 of T lymphocytes, known as invariant natural killer T (iNKT) cells [86]. Glycolipid antigens  
20 (example:  $\alpha$ -galactosylceramide,  $\alpha$ GalCer) are recognized by iNKT cells with the presentation  
21 by CD1d, an MHC-I-like molecule (Figure 4). The CD1d molecule is nonpolymorphic,  
22 therefore, it allows the certain ligand  $\alpha$ GalCer to fit the lipid chain into cavity of CD1d and  
23 leave the TACA moiety outside to be recognized by TCR on iNKT cell [87]. After being  
24 activated, iNKT cells rapidly release a diverse array of Th1 cytokines (e.g. interferon- $\gamma$ , IFN $\gamma$ )  
25 and Th2 cytokines (e.g. IL-4) to modulate natural killer (NK) cells and B cells responses,



1 respectively [88, 89]. Besides, the generated cytokines could also induce activation cascades  
2 of other immunocompetent cells, such as CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, DC cells. Thus,  
3  $\alpha$ GalCer has been called a “universal helper” due to its powerful self-adjuvant properties [35].  
4 **Figure 4.** Pathway of immunomodulation by invariant natural killer T (iNKT) cells. The dendritic cells (DCs)  
5 present the antigen GM3 by the  $\alpha$ GalCer receptor CD1d on DCs (structure of  $\alpha$ GalCer shown in the top left). After  
6 the TCR ligation, the iNKT cells are activated and then secrete multiple Th1- and Th2-type cytokines, such as  
7 IFN $\gamma$  and IL-4, influencing the activation of natural killer (NK) cells and B cells, respectively.

## 8 **5. Strategies to boost immune response of GM3 vaccines**

9 Because of immunological tolerance towards tumor antigens, the immunogenicity of  
10 self-antigens, particularly small carbohydrates and glycopeptides in synthetic vaccines, is  
11 insufficient to provoke a robust immune response. Hence, besides the specificity, vaccines  
12 must possess sufficient potency to overcome tolerance and reestablish surveillance. The most  
13 direct and efficient approach is to couple GM3 to highly immunogenic carriers. Another  
14 method to break immune tolerance to GM3 involves employing modifications onto GM3 or  
15 structural analogs. These GM3 derivatives could potentially exhibit heightened  
16 immunogenicity and provoke a vigorous antibody production which may have  
17 cross-reactivity with native GM3. Here, an introduction will be provided from both the  
18 aspects of the varied immunogenic carriers and GM3 derivatives.

### 19 **5.1 Exploit more effective carriers**

20 In the context of sugar-based vaccine carriers, the primary consideration revolves around  
21 the immunogenicity of the carrier itself, including the antigenic epitope sequences of  
22 heterologous protein carriers, or the patterned ligands recognized by the human humoral  
23 immune system of the non-protein carriers. In addition to this, the size effect is also taken into  
24 account in vaccine delivery. Studies have demonstrated that vaccines with larger size exhibit  
25 enhanced interactions with APCs, such as those present on whole cells, virus-like particles,  
26 and liposomal delivery systems [90].

#### 27 **5.1.1 Protein carriers**

28 Covalently conjugating TACAs with immunogenic proteins has been successfully applied  
29 to develop anticancer vaccines. Over the years, carrier proteins like KLH, tetanus toxoid

1 (TTox), cross-reactive material 197 (CRM197), bovine serum albumin (BSA), human serum  
 2 albumin (HSA) and ovalbumin (OVA) have been extensively utilized in glycoconjugate  
 3 vaccines (Table 1). The covalent conjugation of TACAs with these carriers has proven to  
 4 significantly enhance the immune response [91-93].

5 Table 1. Common Immunogenic Protein Carriers for TACAs

	<b>Molecular Weight</b>	<b>Glycosylation</b>	<b>Multimerization</b>	<b>Amino acid count.</b>	<b>Lysine composition</b>	<b>Database entry</b>
<b>KLH</b> (Keyhole limpet Hemocyanin)	392.2 kDa (S-1) <sup>a</sup>	6 <i>N</i> -sites <sup>b</sup>	decamers/ didecamers of	3414	293 (8.58%)	PDB: 4BED UniProt: Q10583
	391.5 kDa (S-2)	3 <i>N</i> -sites	S-1/2	3421	288 (8.42%)	Q10584
<b>CRM197</b> (Detoxified diphtheria toxin)	61.6 kDa	no	no	567	41 (7.23%)	PDB: 4AE0 UniProt: P00588
<b>TTox</b> (Tetanus toxoid)	150.7 kDa	no	no	1315	114 (8.67%)	PDB: 5N0C UniProt: P04958
<b>HSA</b> (Human serum albumin)	69.4 kDa	23 <i>N</i> -sites	no	609	64 (10.51%)	PDB: 1AO6 UniProt: P02768
<b>BSA</b> (Bovine serum albumin)	69.3 kDa	1 <i>N</i> -sites	no	607	65 (10.71%)	PDB: 3V03 UniProt: P02769
<b>OVA</b> (ovalbumin)	42.9 kDa	1 <i>N</i> -sites	no	386	32 (8.29%)	PDB: 1JTI UniProt: P01012

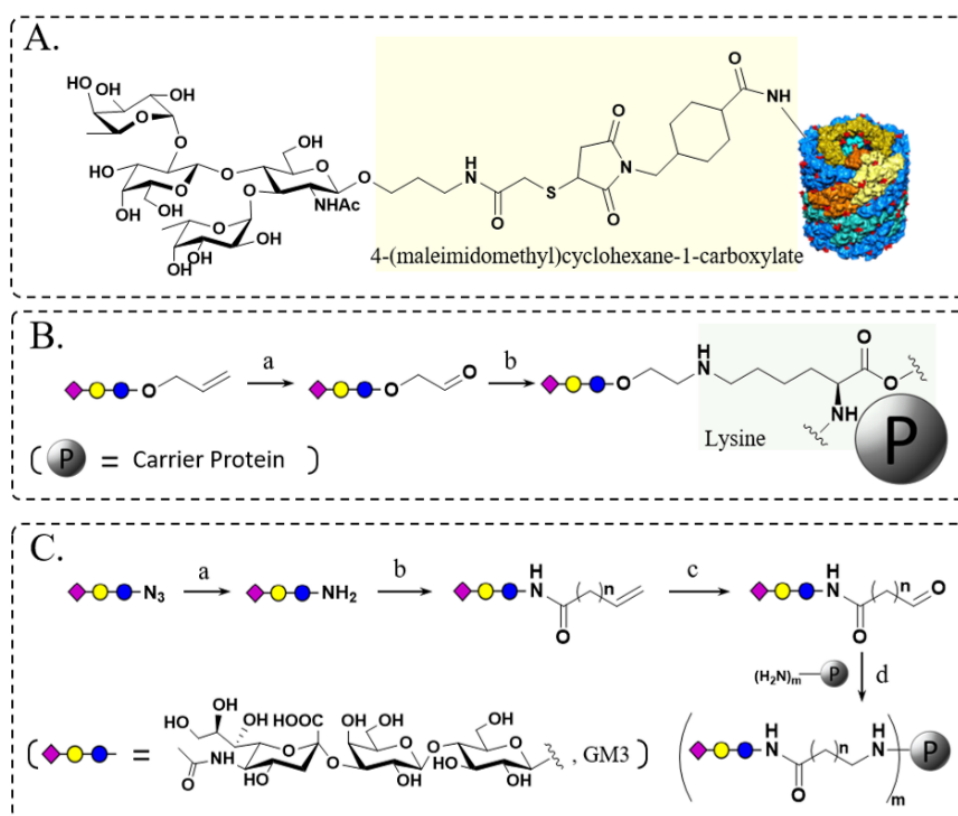
6 <sup>a</sup> (S-1/2): two subunits of KLH sharing around 60% identity at the protein level;

7 <sup>b</sup> *N*-site: number of identified *N*-glycosylation sites provided by the database UniProt (<https://www.uniprot.org/>).

8 In order to make appropriate sugar-protein coupling, the linker holds significant importance.  
 9 They increase the efficiency of conjugations by lessening the steric hindrance. Besides, the  
 10 proper linkers provide more flexible rotation and enhance the exposure of antigenic epitopes  
 11 on the surface of candidates [94]. A number of methods, such as reductive amination,  
 12 photocatalyzed thiol-ene reaction, azide-alkyne Click reactions and others, have been devised  
 13 to facilitate the glycoconjugation of selected molecular and biomolecular entities [95].  
 14 However, some structurally complicated linkers may induce the production of anti-linker  
 15 antibodies, which can suppress the IgG antibody response against TACAs. Boons and  
 16 colleagues found that a Lewis<sup>y</sup> antigen conjugated to KLH using a rigid cyclic  
 17 4-(maleimidomethyl) cyclohexane-1-carboxylate linker suppressed the IgG antibody response  
 18 against the Lewis<sup>y</sup> epitope (Figure 5A). A thorough analysis of sera through enzyme-linked  
 19 immunosorbent assay (ELISA) confirmed a robust IgG antibody response going against the  
 20 linker region. Therefore, selecting a structurally simple and flexible linker can avoid this



1 problem. A common strategy is to introduce an ethylenic bond onto the reducing end  
 2 glycosidic linkage of oligosaccharides, followed by oxidation to form an aldehyde [96].  
 3 Subsequently, the oligosaccharide is coupled to the lysine side chain of the carrier protein  
 4 through a reductive amination reaction (Figure 5B), and it has been widely applied to the  
 5 conjugation of GM3. Zheng et al. loaded 6.6~11.0% and 2.7~5.5% ( $W(\text{carbohydrate}) / W(\text{conjugate}) * 100\%$ ) of GM3 onto KLH and BSA, respectively. The sera obtained from mice  
 6 immunized with the KLH-conjugated vaccines showed a significant titer of both IgM and IgG  
 7 antibodies against GM3-HSA in ELISA assays. This indicates the occurrence of a  
 8 GM3-specific immune response without other signals from the carrier KLH [97]. Guo's team  
 9



10 invented a convergent synthesis method for the azido derivative of GM3, along with its  
 11 convenient coupling to proteins using an acyl linker (Figure 5C). This coupling strategy was  
 12 efficient, with 10.8~13.5% and 11.9~17.5% of carbohydrate loading onto KLH and HSA,  
 13 respectively. It was later confirmed that those KLH conjugates of GM3 and three derivatives,  
 14 coupled using this method, induced a good GM3-specific antibodies in immunized mice after  
 15 35 days [98, 99].

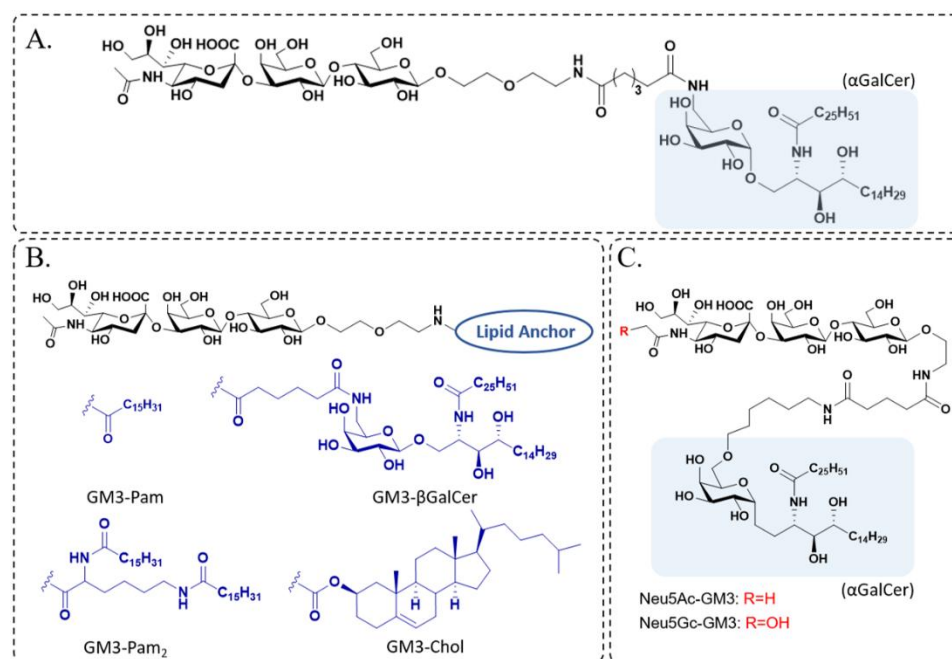
1 **Figure 5.** Representative linkers and general strategies for glycoconjugation to protein carriers. **A.** an  
2 example of a rigid cyclic 4-(maleimidomethyl) cyclohexane-1-carboxylate linker illustrating that  
3 complex linker structures will compete for immune responses, induce linker-specific antibodies and  
4 suppress TACA-specific antibody production; **B.** A general strategy for glycoconjugation with alkenyl  
5 glycosides. Reagents and conditions: (a) O<sub>3</sub> ozonolysis; (b) reductive amination reaction using  
6 NaBH<sub>3</sub>CN. Details seen in Ref. 97; **C.** An azido group on reducing end of GM3 for neoglycoprotein  
7 coupling strategy. Reagents and conditions: (a) selective reduction of the azido group; (b)  
8 (CH<sub>2</sub>=CH(CH<sub>2</sub>)<sub>n</sub>CO)<sub>2</sub>O; (c) O<sub>3</sub> ozonolysis; (d) reductive amination using NaBH<sub>3</sub>CN. Details seen in  
9 Ref. 98.

10 Several highly immunogenic recombinant proteins have also been employed in the  
11 development of GM3 neoglycoconjugates and validated for anti-tumor vaccine activity [100].  
12 For instance, Stepanenko's team utilized flagellins from Gram-positive bacteria (52.5 kDa,  
13 containing 30 lysine residues) to couple with GM3, with an average of 11 GM3 molecules  
14 coupled to each of this neoglycoconjugate. The C57Bl/6J mice were administrated with 2  
15 doses in an interval of two weeks. ELISA testing revealed a successful evocation of anti-GM3  
16 IgG and IgM antibody production, which is similar to those induced by the GM3-KLH  
17 vaccine. Furthermore, dendritic cells pulsed with GM3-flagellins or GM3-KLH were used for  
18 mouse immunization, and the flow cytometry analysis showed that this neoglycoconjugate  
19 induced stronger MHC-II expression and CD80/CD86 differentiation [101, 102].

#### 20 5.1.2 Agonist for iNKT cells

21 As an iNKT cell agonist, αGalCer has been utilized in the development of a self-adjuvant  
22 vaccine based on GM3. In a study conducted by Yin et al., GM3 was covalently coupled to  
23 αGalCer (Figure 6A). Besides, loaded with 4 different lipid linkers, GM3-lipids  
24 non-covalently self-assembled with α-GalCer to form vaccine candidates (Figure 6B). Those  
25 chemical synthesized vaccines were assembled with  
26 1,2-distearoyl-sn-glycero-3-phosphocholine and cholesterol to form liposomes. With  
27 immunization to BALB/c mice, these liposome vaccines were evaluated according to  
28 anti-GM3 antibody production as well as the complement dependent cytotoxicity assay (CDC)  
29 against the B16F10 cell line. The results demonstrated that, the GM3-αGalCer covalent  
30 vaccine candidate can elicit a high level of anti-GM3 IgG production, demonstrating potent  
31 binding affinity to cancer cells and effective activation of the complement system. This  
32 underlined the importance of covalent linkers in enhancing cancer vaccine efficacy. Although

1 GM3 non-covalently conjugated to  $\alpha$ GalCer through four lipid linkers had higher GM3  
 2 loading ratios to the liposome vaccines in this study, the results confirmed that these  
 3 non-covalently coupled formulations were not as effective as GM3- $\alpha$ GalCer [103].



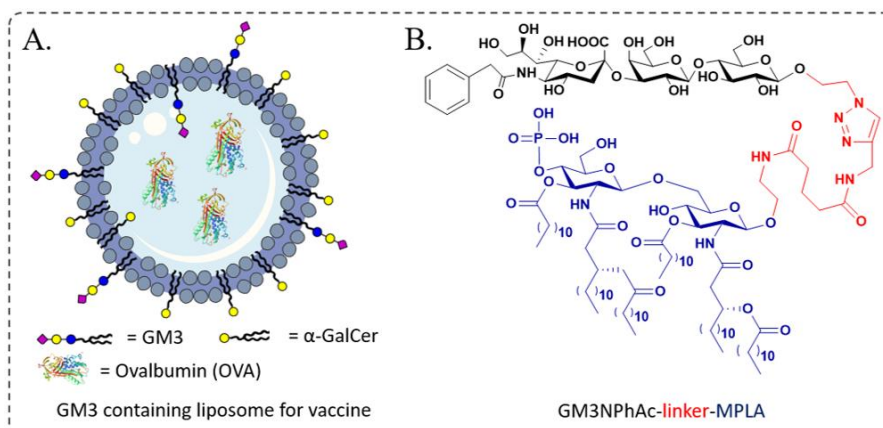
4 **Figure 6.** GM3- $\alpha$ GalCer vaccines, of which the  $\alpha$ GalCer (in blue frame) performs the role of iNKT  
 5 agonist and also an anchor for composition of liposome vaccine. **A.** A covalent GM3- $\alpha$ GalCer  
 6 (self-adjuvant) vaccine candidate; **B.** Four non-covalent GM3-lipid/ $\alpha$ GalCer vaccine candidates.  
 7 The four compounds were severally mixed with GalCer and self-assembled into liposomal  
 8 structures by amphiphilicity. Details of A and B seen in Ref. 103. **C.** Two GM3- $\alpha$ GalCer  
 9 vaccine candidates. Details seen in Ref. 104.

10 Romanò et al. reported a straightforward and efficient chemical synthesis method,  
 11 covalently coupling Neu5Ac-GM3 and Neu5Gc-GM3 with  $\alpha$ GalCer through a linker to form  
 12 liposome vaccines (Figure 6C). These uniform liposomes with a controlled size of  
 13 approximately 200nm were subjected to *in vitro* and *in vivo* immunological tests on iNKT  
 14 cells and C57BL/6 mice, respectively. APCs pulsed with these vaccines were co-cultured with  
 15 iNKT cells for 48 hours, a significant increase in the cytokines IL-4 and IFN- $\gamma$  was observed  
 16 in the *in vitro* culture medium. Similarly, murine immunization experiments also  
 17 demonstrated that both vaccines could successfully activate the cytokines IL-4, IFN- $\gamma$ , and  
 18 IL-12p70, thereby recruiting Th1 and Th2 T-cell responses. Furthermore, they successfully  
 19 activated B-cell responses, resulting in the production of anti-GM3 antibodies. The two sera  
 20 induced by Neu5Gc-GM3 and Neu5Ac-GM3 vaccines, respectively, exhibit cross-reactivity

1 against both antigens [104]. This phenomenon is not surprising in TACA vaccine research  
2 [105, 106].

3 In addition to coupling the trisaccharide moiety of GM3 to an immunogenic carrier, the  
4 complete GM3 ganglioside can also activate immune responses in the form of liposomes.  
5 Haan's team reported a GM3 vaccine study based on the iNKT cell pathway. In this study,  
6 GM3 ganglioside,  $\alpha$ GalCer and OVA were mixed to form liposomes and used for mouse  
7 immunization (Figure 7A). Using CD169<sup>+</sup> macrophage-deficient mice as a control, it was  
8 confirmed that this liposomal vaccine could activate downstream iNKT cells through antigen  
9 presenting by CD169<sup>+</sup> macrophages. Furthermore, the liposome immunization group  
10 exhibited stronger cytokine production, CD8<sup>+</sup> T-cell activation and B-cell responses (antibody  
11 production) compared to the GM3 ganglioside plus adjuvant group. This study suggests that  
12 GM3/ $\alpha$ GalCer liposome can serve as a platform promoting interactions between different  
13 immune cell populations, leading to robust adaptive immunity and emerging as a promising  
14 anticancer vaccination strategy [107]. Following this, the team reported a more mature GM3  
15 antigen-containing liposome vaccine platform: the incorporation of TLR4 or TLR7/8 ligands  
16 promotes dendritic cell maturation and phagocytosis, leading to a stronger T-cell response  
17 [108].

18 In recent years, researches indicated that Neu5Gc-GM3 can bind to the CD1d receptor in  
19 both humans and mice [109, 110]. Furthermore, a study that published in 2016 demonstrated  
20 that Neu5Gc-GM3 can be presented by CD1d of B cells and activate iNKT cell responses.  
21 This discovery of Neu5Gc-GM3 serving as an iNKT cell agonist unveils an important  
22 immunological mechanism, expanding the potential functions of GM3 in immunotherapy and  
23 vaccine development [111].



1 **Figure 7. A.** A model of liposome formed by GM3 and  $\alpha$ GalCer for vaccine delivery. Details  
2 seen in Ref. 107; **B.** A GM3 derivative, GM3NPhAc, conjugated with a toll-like receptor (TLR)  
3 ligand, mono-phosphoryl lipid A (MPLA) to form a vaccine candidate. Details seen in Ref. 122.

#### 4 5.1.3 Pattern recognition receptors (PRRs) as carriers or co-delivery with liposomes

5 Toll-like receptors (TLRs) are a class of transmembrane PRR proteins typically found on  
6 sentinel cells like macrophages and DCs. These receptors identify structurally conserved  
7 molecules originating from microbes. Once these TLR ligand molecules encounter physical  
8 barriers, they are firstly recognized by TLRs, initiating immune cell responses. Consequently,  
9 TLR ligands can be employed as carriers for the development of TACA based anticancer  
10 vaccines [112].

11 In early stages, researchers hydrolyzed the bacterial *N. meningitidis*, obtaining its cell wall  
12 components known as *Neisserial* proteoliposomes. This is an immunogenic proteosome  
13 capable of activating PPRs and self-assembling into proteoliposomes [113]. In order to boost  
14 the immunogenicity, GM3 was combined with *Neisserial* proteoliposomes to produce very  
15 small size proteoliposomes (VSSP). When utilized as a vaccine emulsified with an oil-based  
16 adjuvant, the GM3/VSSP triggered robust anti-GM3 antibody titers (both IgM and IgG) in  
17 mice, chickens, and monkeys [13]. Furthermore, GM3/VSSP administration demonstrated  
18 efficacy in protecting against melanoma, as evidenced with highly anti-GM3 specific  
19 antibody production and anti-B16 complement-mediated cytotoxicity in C57Bl/6 mice [114,  
20 115]. Another research confirmed that the GM3/VSSP vaccine induces dendritic cell  
21 maturation and presentation of GM3 through TLR2-mediated signal transduction, activating  
22 the release of IL-6 and IFN- $\gamma$  cytokines [116]. In a Phase I clinical trial involving 26  
23 participants with metastatic melanoma, where GM3/VSSP was utilized as an anti-melanoma  
24 vaccine, 44% of the subjects exhibited the production of anti-GM3 IgM in their serum.  
25 Moreover, these sera were observed to elicit responses against melanoma cell lines and biopsy  
26 tissues. A robust secretion of IFN- $\gamma$  cytokine was detected in the sera of all participants, with  
27 one sample identified as GM3-specific [117].

28 With the development of synthetic chemistry, the core structures derived from bacterial  
29 LPS have been gradually elucidated and successfully synthesized [118, 119]. The  
30 mono-phosphoryl lipid A (MPLA) is 1-*O*-dephosphorylated lipid A, which is a most

1 representative bacterial LPS structure. MPLA exhibits potent immunostimulatory properties  
2 and serve as effective vaccine adjuvants. The immunostimulatory activity of MPLAs is  
3 ascribed to their capacity to initiate cytokine cascades by interacting with toll-like receptor 4  
4 (TLR4). This interaction can trigger antigen presentation, activate T-helper (Th) cells, and  
5 consequently induce T cell-mediated immunity [120]. Guo's team identified a highly  
6 immunogenic GM3 derivative trisaccharide GM3NPhAc in their former study [99, 121].  
7 Based on this, the *N. meningitidis* MPLA was used as vaccine carrier to create a  
8 glycoconjugate and administered to mice (Figure 7B). The results revealed that this vaccine  
9 elicited an effective immune response to GM3NPhAc without the use of an external adjuvant,  
10 particularly leading to the generation of carbohydrate-specific IgG3 subtype antibodies.  
11 Interestingly, for one test group in this study was utilized Titermax Gold as adjuvant, but it  
12 was observed that the adjuvant did not enhance the antibody production; instead, it appeared  
13 to inhibit the GM3NPhAc-specific immune response. This provides insights for selecting  
14 carriers and immunization strategies in the development of GM3 anti-tumor vaccines [122].

15 In addition to serving as an antigen, GM3 can also be in co-delivered with PRRs by  
16 liposomes and facilitate macrophage to capture the liposome vaccines. CD169<sup>+</sup> splenic  
17 macrophages efficiently capture particulate antigens from the blood and transfer them to  
18 dendritic cells for downstream immune responses. In a study published in 2020 by Haan's  
19 group, it was reported that the inclusion of GM3 in liposomes enhances the engulfment by  
20 CD169<sup>+</sup> splenic macrophages, thereby presenting antigens to activate CD8<sup>+</sup> and CD4<sup>+</sup> T cell  
21 responses [123]. The team reported new results two years later, with a study that they  
22 incorporated MPLA (TLR4 ligand), 3M-052 (TLR7/8 ligand) and an inflammasome stimulus  
23 either alone or in combination into GM3 liposomes. Both *in vitro* and *in vivo* results  
24 confirmed that MPLA-containing GM3 liposomes are effective vectors to induce DC  
25 maturation and CD4<sup>+</sup>/CD8<sup>+</sup> T cell priming. Although these studies did not focus on whether  
26 the immunized animals produced a specific anti-GM3 response, the activation of  
27 macrophages by GM3 liposomes itself provides a promising strategy for the development of  
28 anti-tumor vaccines [108].

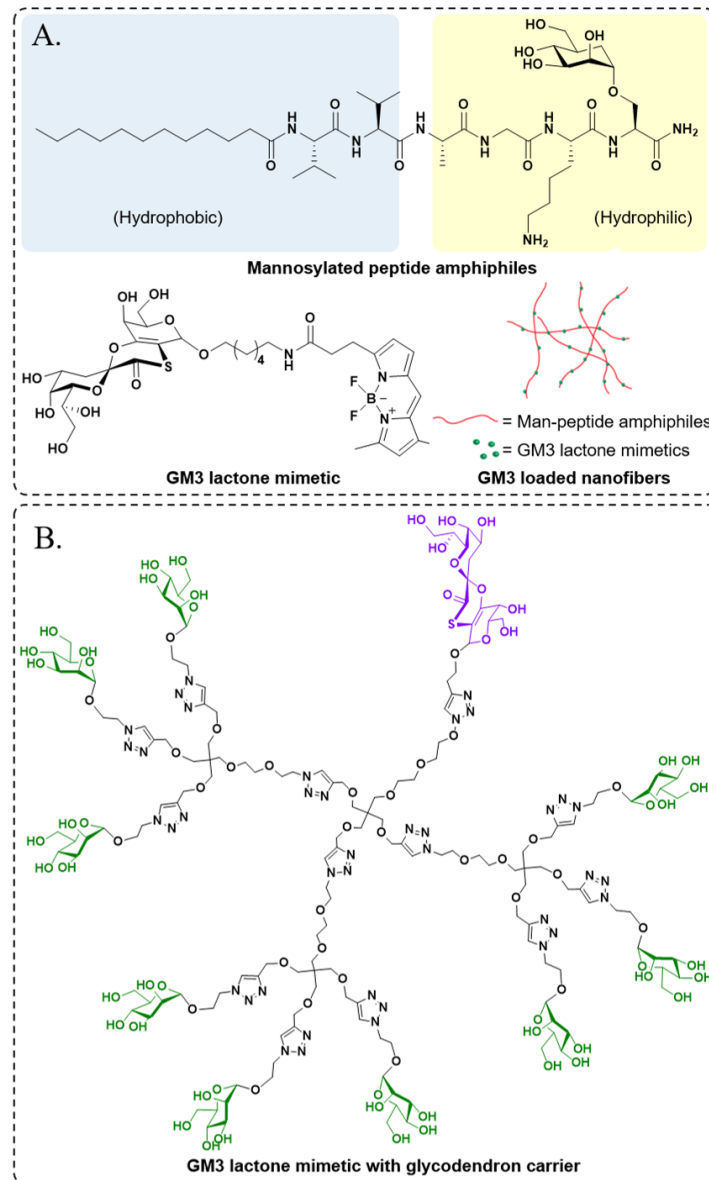
#### 29 5.1.4 Other carriers

1 Other emerging highly immunogenic biomaterials have also been developed as carriers for

1 coupling TACAs to prepare vaccines. For instance, Virus-like particles (VLPs) are complex



1 protein structures that mimic the envelopes or capsids of authentic native viruses but lack the  
 2 viral genome, falling within a similar particle size range (0.02–0.06 $\mu$ m) [124]. Taking  
 3 advantage of this more natural immunogenic structure, VLPs have been developed for TACA  
 4 delivery. Huang's group utilized a cowpea mosaic virus (CPMV) capsid as the immunogenic  
 5 carrier for conjugation with Tn antigen. The resulting Tn-CPMV was administered to mice,



6 generating high total antibody titers and high IgG titers specific to Tn antigen [125]. In  
 7 another study, a bacteriophage Q $\beta$  conjugated with SLewis<sup>a</sup> antigen was used to immunize  
 8 mice. Compared to the control group immunized with SLewis<sup>a</sup>-KLH, the sera from  
 9 SLewis<sup>a</sup>-Q $\beta$  immunized mice exhibited higher anti-SLewis<sup>a</sup> antibody titers, which remained  
 10 detectable at high levels even after 379 days [126].

11 **Figure 8. A.** Nanofibers loaded with GM3 lactone mimetic as a vaccine candidate. Details

1                    seen in Ref. 127; **B.** Structure of a glycodendron consisting of nine mannoses (in green) for  
2                    DC targeting and one GM3 lactone mimetic (in purple) as antigen. Details seen in Ref. 128.

3            In recent years, with highly regulated dimensions, controllable volumes and abundant  
4 surface modification sites, nanomaterials have also been developed as chemical carriers for  
5 TACA based vaccines, e.g., gold nanoparticles, nanofibers and other chemical scaffolds.  
6 Nativi's group synthesized a thioether-bridged mimetic of GM3 lactone for antigen coupling  
7 with various nanomaterial carriers [51, 127-129]. They synthesized a mannose-containing  
8 peptide amphiphiles, which can spontaneously assemble into nanofibers in aqueous solution  
9 with diameters on the order of 9 ~11 nm and lengths reaching several micrometers. These  
10 nanofibers were then mixed with a hydrophobic fluorescent GM3 lactone mimetic, allowing  
11 the hydrophobic portion to be inserted into the interior of the nanofiber while exposing the  
12 hydrophilic antigenic portion outside (Figure 8A). The abundant mannose on this nanofiber  
13 carrier serves as a ligand for DC-SIGN on DCs. By *in vitro* experiments, it was confirmed  
14 that these nanofibers could activate DCs to phagocytize the antigens of GM3 lactone mimetic.  
15 Furthermore, the over-expression of CD86, CD83 and MHC-II on DCs indicated that these  
16 nanofibers could also promoted the maturation of DCs [127]. Similarly, they synthesized a  
17 bifunctional multivalent glycodendron consisting of nine mannose residues for DC targeting  
18 and one residue of the antigen, GM3 lactone mimetic (Figure 8B). Immunological assays  
19 revealed that this glycodendron is able to trigger the activation of human immature DCs and  
20 promote the proliferation of T lymphocytes in a mixed lymphocyte reaction [128].

## 21        **5.2 Chemical modifications on GM3**

22        Protein-carbohydrate interactions present peculiarities compared to general protein-rigid  
23 ligand and protein-peptide interactions. Firstly, many carbohydrates exhibit extreme  
24 flexibility and contain numerous hydroxyl groups, resulting in the formation of extensive  
25 hydrogen-bonding networks that significantly influence the overall binding free energy.  
26 Secondly, a notable characteristic of protein-carbohydrate interactions is the formation of  
27 essential CH- $\pi$  contacts between the aromatic side chains of the protein and the C-H bonds of  
28 the carbohydrates, particularly on their hydrophobic surfaces [130, 131]. Therefore, the  
29 specificity in carbohydrate-antibody interactions is more tolerant. Taking GM3 as an example,

1 numerous studies in recent years have demonstrated that chemically modified GM3  
2 derivatives exhibit enhanced immunogenicity during the immune response, and the resulting  
3 antibodies show varying degrees of cross-reactivity to natural GM3, which means the  
4 antibodies induced by derivatives could fight against the GM3 containing cancer cells [132].  
5 This provides an important strategy for the development of GM3 derivatives as anti-tumor  
6 vaccines.

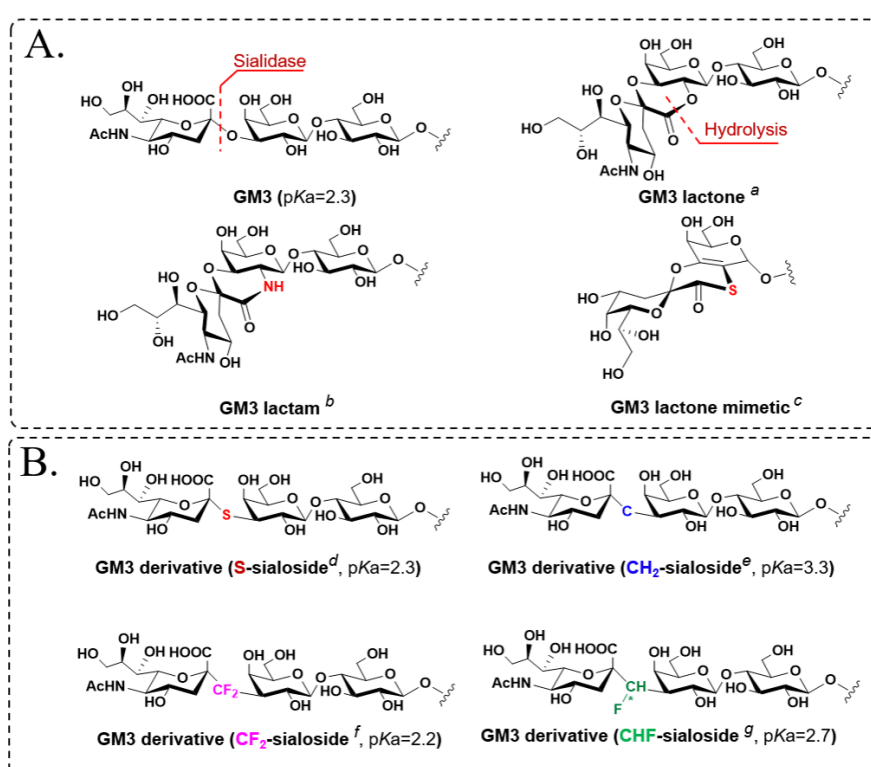
#### 7 5.2.1 GM3 lactone and mimetics

8 As early as 1987, Hakomori's team found that a monoclonal antibody M2590 reacts not  
9 only with GM3 but also with GM3 lactone (Figure 9A-a), and the antibody exhibits a  
10 significantly higher binding affinity to GM3 lactone compared to GM3. Based on this finding,  
11 they immunized BALB/c mice separately with GM3 and GM3 lactone. The results showed  
12 that GM3 lactone is more immunogenic, triggering a stronger production of antibodies which  
13 were able to bind to both GM3 and GM3 lactone demonstrating cross-reactivity to both  
14 antigens [43]. Subsequently, they immunized C57BL/6 mice with GM3 lactone coated on  
15 *Salmonella minnesotae*, establishing hybridoma DH2 which secretes IgG3 subtype antibodies.  
16 Those antibodies were proven to preferentially react with GM3 lactone over GM3, but  
17 inhibited melanoma cell growth *in vivo* and induced antibody-dependent cytotoxicity *in vitro*  
18 [133].

19 While GM3 lactone exhibits higher immunogenicity compared to natural GM3, the  
20 stability of the lactone's ring structure remains a concern. To overcome the hydrolytic  
21 instability of the lactones, Magnusson and colleagues developed a GM3 lactam (Figure 9A-b)  
22 which should be hydrolytically stable *in vivo* while still being conformationally similar to the  
23 corresponding lactone structure. Immunization of BALB/c mice with a GM3 lactam-BSA  
24 conjugate, followed by the generation of hybridomas, resulted in a significant number of  
25 clones specific to GM3 lactam. Intriguingly, the majority of these clones exhibited  
26 recognition of GM3 lactone but not GM3 [134].

27 For the same purpose of enhancing hydrolytic stability, Nativi's team synthesized a  
28 thioether-bridged mimetic of GM3 lactone (Figure 9A-c) and conjugated it with various  
29 carriers for vaccine biological activity testing [135]. In their work published in 2010, this

1 mimetic was conjugated to KLH and used to immunize BALB/c mice. A hybridoma  
 2 producing the strongest anti-GM3 antibody was selected, and further analysis showed its  
 3 isotypes were identified as IgM and IgG3. Immunocytochemistry results demonstrated that  
 4 polyclonal antibodies from the immunized mouse serum bound to HCT116 cells (human  
 5 colon tumor cells overexpressing the GM3 antigen) [51]. Subsequently, in 2014 and 2017,  
 6 they respectively reported the conjugation of this mimetic of GM3 lactone to non-protein  
 7 carriers glycodendron and nanofibers as vaccine candidates, successfully activating DC  
 8 immune response.



9 **Figure 9.** A. GM3 lactone and mimetics (red dashed lines indicate chemical bond susceptible to sialidase  
 10 or hydrolysis). Details seen in <sup>a</sup>Ref. 43, 133, <sup>b</sup>Ref. 134, and <sup>c</sup>Ref. 135; B. Sialidase-resistant analogues of  
 11 GM3. Details seen in <sup>d</sup>Ref.137 and <sup>e-g</sup>Ref. 140.

### 12 5.2.2 Modifications on the glycosidic bond

13 Sialidases are enzymes that can cleave sialic acid glycosidic bonds. For example, the  
 14 upregulated sialidase NEU3 in many malignant cancers can cleave *O*- $\alpha$ -2,3 glycosidic bonds  
 15 and remove sialic acid from GM3. The exogenous GM3 introduced as anticancer medicines  
 16 or vaccines needs to avoid degradation by these glycosidases and maintain its intact structure  
 17 to exert biological activity. Therefore, many studies have developed representative

1 sialidase-resistant active analogues targeting sialic acid glycosidic bonds.

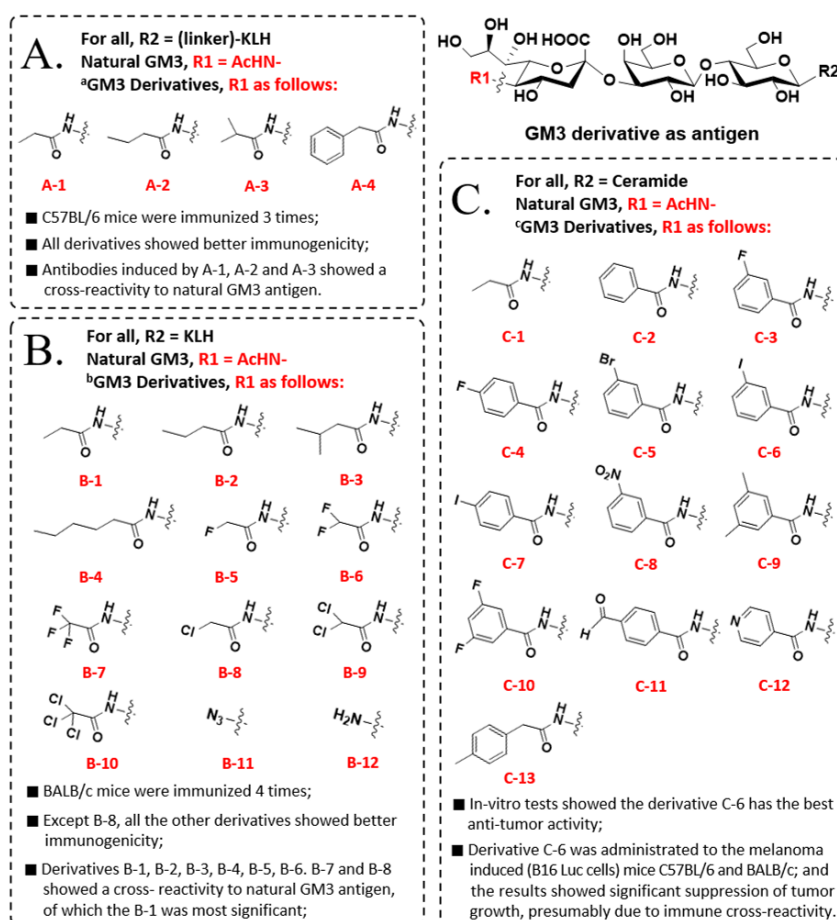
2 One direct method involves substituting the susceptible *O*-sialoside bond in  
3  $\alpha$ -2,3-sialylgalactose with an alternative linkage, while minimizing structural alterations to the  
4 cyclic sialic acid moiety. Considering this, *S*-Sialosides (Figure 9B-d) was taken into tests,  
5 which exhibit greater flexibility compared to *O*-sialosides due to their longer C-S bond,  
6 leading to decreased steric and stereo-electronic effects [136]. Bundle's group synthesized this  
7 *S*-sialoside substituted GM3 analogue and loaded it onto TTox for immunological assays.  
8 Five BALB/c mice were immunized with three doses of *S*-GM3-TTox glycoconjugates on  
9 days 1, 21 and 28, and serum antibodies were tested on day 38. The results showed a strong  
10 antigen-specific IgG response when immune sera were reacted against *S*-GM3. Additionally,  
11 three out of five mice showed a cross-reactivity against natural GM3, with good antibody  
12 titers ranging from  $10^3$  to  $10^4$ . Analysis of IgG subtypes indicated that this *S*-GM3  
13 glycoconjugate vaccine successfully activated the T cell-dependent immune pathway [137,  
14 138]. The analysis of molecular structures suggests that while the C-S bond is longer than the  
15 C-O bond, the C-S-C angle is significantly smaller than the C-O-C angle. *S*-glycosidic bonds,  
16 being less constrained than their *O*-glycoside counterparts, can more readily access the higher  
17 energy anti conformation in both unbound and bound states. The replacement of oxygen with  
18 sulfur atom leads to longer bonds and weaker stereo-electronic effects, allowing for  
19 substantially greater flexibility. This flexibility poses a potential challenge to the use of  
20 *S*-glycoside immunogens [139]. According to the *in vivo* experimental results, it appears that  
21 this flexibility introduces some randomness but still holds significant inspiring implications.

22 Another category of glycosidic modification for sialidase-resistance of GM3 analogue that  
23 has attracted attention is *C*-glycosides. Harai's group has recently incorporated four types of  
24 *C*-sialoside linkages ( $\text{CH}_2$ -,  $\text{CF}_2$ -, (*R*)-CHF-, and (*S*)-CHF-sialosides, shown in Figure 9B-e, f  
25 and g) into the synthesis of GM3 using a series of strategies [140]. They first assessed the  
26 impact of each glycoside on the pKa value of sialic acid in these *C*-sialosidic analogues. The  
27 results showed that the  $\text{CH}_2$ -sialoside could increase the pKa of sialic acid (from 2.3 to 3.3,  
28 theoretically). Furthermore, in the formation of the *O*- $\alpha$ 2,3 linkage, the lone pairs electrons on  
29 the oxygen atom can achieve a more stable conformation based on the exo-anomeric effect. In  
30 contrast, the  $\text{CH}_2$ -sialoside lacks stereo-electronic effects, resulting in greater conformational

1 flexibility. Therefore, they considered introducing fluorine atoms to maintain the pKa of sialic acid  
 2 acid while obtaining relatively stable conformation of the GM3 analogues through the gauche  
 3 effect. *In vitro* experiments confirmed that these fluorinated C-glycosidic GM3 analogues  
 4 exhibited biological activity similar to natural GM3 by inhibiting the epidermal growth factor  
 5 receptor. Additionally, the (*S*)-CHF-linked GM3 analogue with a stable exo-gauche  
 6 conformation showed the strongest activity in promoting Had-1 cell growth, indicating the  
 7 importance of the specific conformation of GM3 for the biological functions [136]. These  
 8 findings suggest promising avenues for research into the immunological activity of such  
 9 C-glycosidic GM3 analogues in anticancer vaccine development.

### 10 5.2.3 Modifications on sialic acid of GM3

11 Taking advantage of the potential cross-reactivity in antibody-carbohydrate reactions,  
 12 researchers have introduced chemical modifications to sialic acid in order to disrupt the  
 13 immunotolerance and immunosuppression of GM3. Guo's team replaced the acetamido group  
 14 of sialic acid and synthesize four GM3 derivatives A-1~A-4 as shown in Figure 10A. The  
 15 immunological properties of the GM3 derivative-KLH glycoconjugates were assessed in  
 16 C57BL/6 mice with 3 immunizations at day 1, day 27 and day 35. By analyzing the serum  
 17 collected on day 35 using ELISA, the results showed that GM3-KLH only activated an IgM  
 18 response, while the other four GM3 derivative-KLH conjugates not only activated an IgM



1 response but also elicited a stronger IgG response. Overall, glycoconjugate A-4 exhibited the  
2 highest immunogenicity, followed by A-3, A-2, and A-1. However, only sera from mice  
3 immunized with A-1, A-2, and A-3 showed cross-reactivity with natural GM3. These findings  
4 suggest that larger structural modifications of sialic acid (e.g. A-4) can improve  
5 immunogenicity, but the induced antibodies may not react against natural GM3 due to the  
6 significant differences in the antibody binding pockets [99].

7 **Figure 10.** GM3 derivatives with *N*-modification on sialic acid. Details seen in <sup>a</sup>Ref. 99, <sup>b</sup>Ref. 97 and <sup>c</sup>Ref. 142.

8 Taking lessons from their previous work, Ye et al. found that the carboxylic acid and sugar  
9 type are more sensitive to maintain the cross-recognition of antibodies induced by TACA  
10 derivative and should be kept intact [141]. They focused on maintaining the carboxyl group of  
11 sialic acid unchanged and instead pursued *N*-acyl modification. Twelve structurally modified  
12 GM3 derivatives and their KLH conjugates (B-1~B-12 shown in Figure 10B) were designed  
13 and synthesized. BALB/c mice were immunized four times at 2-week intervals with  
14 GM3-KLH or GM3 derivative-KLH glycoconjugates. After the third vaccination, pooled sera  
15 were analyzed for IgG titers against each derivative antigen or natural GM3. Except B-8, all  
16 the other modified GM3 derivatives exhibited stronger immunogenicity, eliciting higher titers  
17 of antibodies against their corresponding GM3 derivative antigens compared to unmodified  
18 GM3. Due to concerns about cross-reactivity, this study found that glycoconjugates B-1, B-2,  
19 B-3, B-4, B-5, B-6, B-7 and B-8 elicited antibodies cross-reacting against GM3. Among them,  
20 B2 and B7 elicited higher titers of IgG antibodies against GM3 compared to the unmodified  
21 GM3-KLH [97].

22 In an article published in 2022, Jiang et al. synthesized 13 *N*-acyl modified GM3  
23 derivatives (C-1~C-13 shown in Figure 10C) and evaluated their anti-tumor activity by  
24 wound healing assays and trans-well assays. Among these derivatives, compound C-6  
25 exhibited significant inhibition of cell migration and invasion, as well as marked anti-tumor  
26 effects in tumor-bearing (epidermoid carcinoma A431 cells) C57BL/6 mouse model.  
27 Subsequent analysis of cancer tissues and serum samples revealed that compound C-6  
28 prompted tumor suppression through inducing tumor necrosis and eliciting an inflammatory  
29 response, indicating that the tumor inhibition induced by C-6 was closely associated with the  
30 carbohydrate cross-reactivity immune response [142]. Although this hypothesis is not entirely

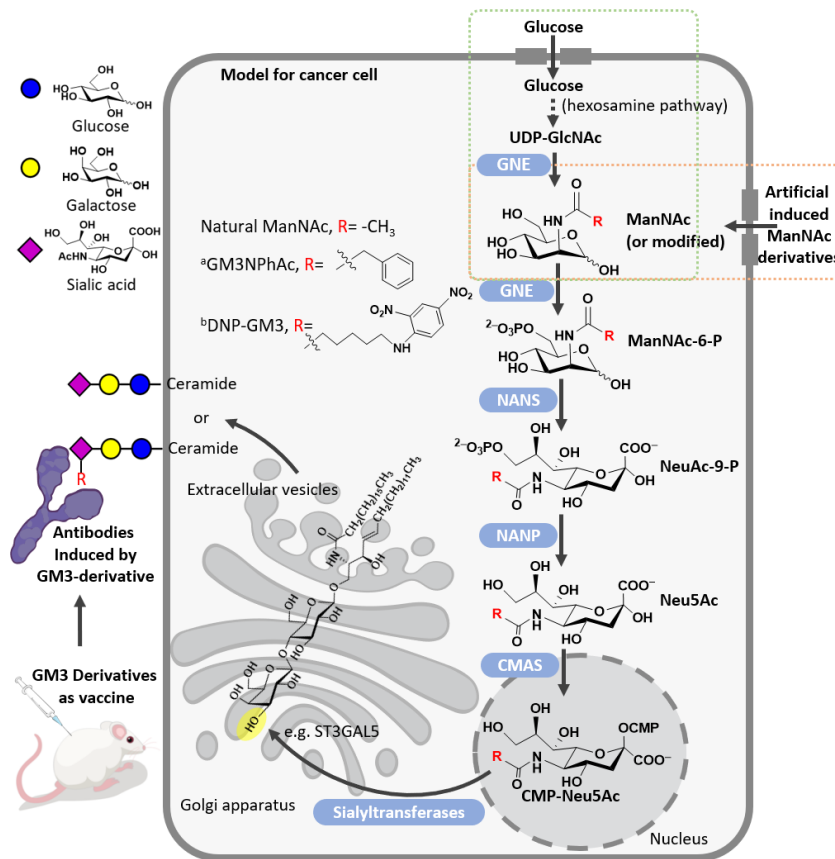
1 consistent with the previous findings conducted by Guo regarding glycoconjugate A-4  
2 (GM3NPhAc-KLH), where the introduction of a large phenyl structure to *N*-acyl moiety of  
3 GM3 did not elicit cross-reactivity against natural GM3, the promising anti-tumor activity  
4 exhibited by compound C-6 still warrants further investigation into immunological pathways.

5 5.2.4 Vaccines based on metabolic glycoengineering of GM3



1 While antibodies generated by GM3 derivatives with more complex modifications (e.g.

1 *N*-phenylacetyl modification on sialic acid) are unlikely to cross-react with natural GM3, the  
 2 significantly increased immunogenicity still attracts researchers to develop anticancer  
 3 immunotherapies using them. Glycoengineering based on sialic acid metabolism could  
 4 provide a highly specific immunotherapy strategy to overcome this issue. In simple terms,  
 5 glycoengineering is a process based on cellular metabolism where sialic acid is synthesized  
 6 from mannose substrates and catalyzed by sialyltransferase to oligosaccharides, which are  
 7 further transferred to the cell surface (Figure 11) [143, 144]. By utilizing this pathway, the  
 8 introduction of chemically modified *N*-acetyl mannose (ManNAc) can introduce more  
 9 corresponding modified sialyl TACAs, including GM3, onto the surfaces of cancer cells. This  
 10 strategy was pioneered by Reutter and colleagues [145]. Further advancements in  
 11 bioorthogonal chemistry, inspired by the groundbreaking work of Bertozzi and colleagues,  
 12 and led to Bertozzi being awarded the Nobel Prize in Chemistry in 2022 [146]. Given that this  
 13 glycoengineering enables the editing of cancer cell surfaces with modified GM3, the  
 14 antibodies activated by the highly immunogenic GM3 derivatives mentioned earlier now have  
 15 a role to play. These robust immune responses can selectively target the glycoengineered



1 cancer cells.

2 **Figure 11.** Vaccines based on metabolic glycoengineering of GM3 and an overview of  
3 mammalian sialic acid metabolism shown in a cellular sketch. Starting from the top, normally  
4 the glucose is taken by cancer cells and converted into ManNAc by following the hexosamine  
5 pathway and GNE (pathway details seen in Ref. 144). If a ManNAc with *N*-modification is  
6 artificially introduced into the cellular environment, it will participate in the subsequent  
7 pathway: catalyzed by enzymes such as GNE, NANS, NANP, and CMAS (pathway details seen  
8 in Ref. 143), ultimately transforming into *N*-modified sialic acid. These sialic acids are then  
9 added onto GM3 by sialyltransferases such as ST3GAL5, and transported to the cell membrane  
10 surface via vesicles. Antibodies and immune responses elicited by immunization with the  
11 corresponding GM3 derivative will specifically recognize these glycoengineered cancer cells.  
12 Details seen in <sup>a</sup>Ref. 147 and <sup>b</sup>Ref 149.

13 The GM3NPhAc (shown as Figure 11. a) glycoconjugate elicited a significantly strong  
14 anti-GM3NPhAc-specific antisera production but the antibodies hardly showed  
15 cross-reactivity to natural GM3 [99]. In a subsequent study, tumor cells (K562, SKMEL-28,  
16 and B16-F0) were incubated with *N*-phenylacetyl-mannosamines (ManNPhAc), resulting in  
17 successful glycoengineering as identified by flow cytometry. These glycoengineered cancer  
18 cells exhibited sensitivity to anti-GM3NPhAc-specific antisera in CDC assays, indicating the  
19 potential of this strategy for developing glycoengineering-based anti-tumor vaccines [147]. In  
20 2015, their team applied this strategy to animal models and obtained promising results.  
21 C57BL/6 mice immunized with GM3NPhAc-KLH-loaded DCs successfully activated T  
22 cell-dependent anti-GM3NPhAc-specific immune responses. One week after the third  
23 immunization, mice were injected with  $5 \times 10^5$  of B16F10 cells followed by daily injections of  
24 ManNPhAc (50 mg/kg/day) for 7 days. The results demonstrated that this strategy  
25 significantly inhibited tumor growth and prolonged the survival of tumor-bearing mice, while  
26 also reducing B16F10 lung metastases [148].

27 In a recent study, GM3 was introduced with a more complex *N*-modification for the aim of  
28 eliciting a stronger immunogenic response through pre-immunization to the anti-modification  
29 and employing glycoengineering strategies for anti-tumor vaccine therapy. A  
30 2,4-dinitrophenyl (DNP)-modified GM3 intermediate (DNP shown as Figure 11. b) was  
31 chemoenzymatically synthesized and conjugated to KLH, conducted by Wu's group. BALB/c  
32 mice pre-immunized with DNP-OVA were subsequently immunized with the  
33 DNP-GM3-KLH glycoconjugates. This approach resulted in a robust and rapid immune

1 response, attributed to the recruitment of anti-DNP antibodies facilitating targeted delivery of  
2 the vaccine construct to APCs. The serum obtained through this immunization strategy  
3 consisted of IgG subtype antibodies without IgM, and exhibited significant CDC against  
4 Man-NHDNP treated B16F10 melanoma cells *in vitro* [149].

## 5 **6. Conclusion**

6 Great progress has been made in the past decades for carbohydrate-based anticancer  
7 vaccine development. As a member of TACAs, the structure and distribution of GM3  
8 molecules make them a prominent target for the development of anti-tumor immunotherapy.  
9 Various strategies have been attempted to exploit its potential in anti-tumor immunotherapy,  
10 including activating the immune response to GM3 hapten through immunogenic carriers,  
11 enhancing immunogenicity by modifying the GM3 glycoside portion then for cross-reactivity  
12 with natural GM3, and introducing labeled GM3 onto tumor cells through  
13 glycoengineering-based bio-orthogonal reactions. These strategies collectively demonstrate  
14 the effectiveness of GM3 in the development of anti-tumor immunotherapy. Based on these  
15 foundations, determining GM3 derivatives with higher immunogenicity and cross-reactivity  
16 to natural antigens, identifying the most cost-effective synthesis methods, selecting the most  
17 efficient delivery carriers, and other related tasks are all essential steps before advancing GM3  
18 into clinical applications.

## 19 **CRedit authorship contribution statement**

20 Dr. Yongmin Zhang, Dr. Marco Terreni and Dr. Matthieu Sollogoub contributed to the  
21 conceptualization, revising, and funding acquisition; Jiaxu Zhang contributed to the original  
22 draft, preparation for the figures and tables, and editing; Fang Liu contributed to the data  
23 collection and revising. All the authors participated in the discussions of the results.

## 24 **Conflict of Interest Statement**

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

## 27 **Acknowledgements**

28 We are grateful for financial support from the CNRS, Sorbonne University, and China

1 Scholarship Council (CSC, No. 202108620047).

2

## References

- [1] F. Liu, H. Liu, M. Sollogoub, Y. Zhang, Recent advances on glycosphingolipid GM3, in: *Carbohydrate Chemistry*, (2020), pp. 276.
- [2] V. Kolyovska, S. Ivanova, D. Drenska, D. Maslarov, R. Toshkova, Role of GM3 ganglioside in the pathology of some progressive human diseases and prognostic importance of serum anti-GM3 antibodies, *BIOCELL*, 45 (2021) 1485--1494. <https://doi.org/10.32604/biocell.2021.016250>
- [3] A. Prinetti, N. Loberto, V. Chigorno, S. Sonnino, Glycosphingolipid behaviour in complex membranes, *Biochimica et Biophysica Acta (BBA) - Biomembranes*, 1788 (2009) 184-193. <https://doi.org/10.1016/j.bbamem.2008.09.001>
- [4] M.S. Toledo, E. Suzuki, K. Handa, S.-i. Hakomori, Cell growth regulation through GM3-enriched microdomain (glycosynapse) in human lung embryonal fibroblast WI38 and its oncogenic transformant VA13, *Journal of Biological Chemistry*, 279 (2004) 34655-34664. <https://doi.org/10.1074/jbc.m403857200>
- [5] T. Sato, Y. Nihei, M. Nagafuku, S. Tagami, R. Chin, M. Kawamura, S. Miyazaki, M. Suzuki, S.-i. Sugahara, Y. Takahashi, A. Saito, Y. Igarashi, J.-i. Inokuchi, Circulating levels of ganglioside GM3 in metabolic syndrome: A pilot study, *Obesity Research & Clinical Practice*, 2 (2008) 231-238. <https://doi.org/10.1016/j.orcp.2008.06.001>
- [6] M. Motohide, Y. Noriko, T. Nario, R. Ludovic, H. Nicolas, P. Brigitte, M. Asami, K. Nozomu, M. Laura, A. Junken, S. Yasushi, S. Sandro, K. Naoko, A. Hiromune, K.K. Mika, K. Yoshinori, I. Kei-ichiro, I. Jin-ichi, M. Yves, I. Kazuhisa, K. Toshihide, Cell density-dependent membrane distribution of ganglioside GM3 in melanoma cells, *Cellular and Molecular Life Sciences (CMLS)*, (2023). <https://doi.org/10.1007/s00018-023-04813-9>
- [7] P. Hersey, O. Jamal, C. Henderson, I. Zardawi, D. D' Alessandro, Expression of the gangliosides GM3, GD3 and GD2 in tissue sections of normal skin, naevi, primary and metastatic melanoma, *International Journal of Cancer*, 41 (1988) 336-343. <https://doi.org/10.1002/ijc.2910410303>
- [8] C. Zheng, R. Huang, T. Bavaro, M. Terreni, M. Sollogoub, J. Xu, Y. Zhang, Design, synthesis and biological evaluation of new ganglioside GM3 analogues as potential agents for cancer therapy, *European Journal of Medicinal Chemistry*, 189 (2020) 112065. <https://doi.org/10.1016/j.ejmech.2020.112065>
- [9] C. Zheng, H. Guan, Y. Liu, Z. Li, T. Bavaro, M. Terreni, M. Sollogoub, J. Xu, Y. Zhang, Chemoenzymatically synthesized ganglioside GM3 analogues with inhibitory effects on tumor cell growth and migration, *European Journal of Medicinal Chemistry*, 165 (2019) 107-114. <https://doi.org/10.1016/j.ejmech.2019.01.016>
- [10] M.V. Gentilini, M.E. Pérez, P.M. Fernández, L. Fainboim, E. Arana, The tumor antigen N-glycolyl-GM3 is a human CD1d ligand capable of mediating B cell and natural killer T cell interaction, *Cancer Immunology, Immunotherapy*, 65 (2016) 551-562. <https://doi.org/10.1007/s00262-016-1812-y>
- [11] A.N. Samraj, H. Läubli, N. Varki, A. Varki, Involvement of a non-human sialic Acid in human

- 1 cancer, *Frontiers in Oncology*, 19 (2014) 33. <https://doi.org/10.3389/fonc.2014.00033>
- 2 [12] B.A.H. Smith, C.R. Bertozzi, The clinical impact of glycobiology: targeting selectins, Siglecs  
3 and mammalian glycans, *Nature Reviews. Drug discovery*, 20 (2021) 217-243. <https://doi.org/10.1038/s41573-020-00093-1>
- 4 [13] F. Estevez, A. Carr, L. Solorzano, O. Valiente, C. Mesa, O. Barroso, G. Victoriano Sierra, L.E.  
5 Fernandez, Enhancement of the immune response to poorly immunogenic gangliosides after  
6 incorporation into very small size proteoliposomes (VSSP), *Vaccine*, 18 (1999) 190-197.  
7 [https://doi.org/10.1016/S0264-410X\(99\)00219-4](https://doi.org/10.1016/S0264-410X(99)00219-4)
- 8 [14] X. Zhou, K. Chi, C. Zhang, Q. Liu, G. Yang, Sialylation: a cloak for tumors to trick the  
9 immune system in the microenvironment, *Biology*, 12 (2023) 832.  
10 <https://doi.org/10.3390/biology12060832>
- 11 [15] H. Yu, J. Shu, Z. Li, Lectin microarrays for glycoproteomics: an overview of their use and  
12 potential, *Expert Review of Proteomics*, 17 (2020) 27-39. <https://doi.org/10.1080/14789450.2020.1720512>
- 13 [16] S.-I. Hakomori, Y. Zhang, Glycosphingolipid antigens and cancer therapy, *Chemistry &*  
14 *Biology*, 4 (1997) 97-104. [https://doi.org/10.1016/s1074-5521\(97\)90253-2](https://doi.org/10.1016/s1074-5521(97)90253-2)
- 15 [17] G. Yang, L. Huang, J. Zhang, H. Yu, Z. Li, F. Guan, Global identification and differential  
16 distribution analysis of glycans in subcellular fractions of bladder cells, *International Journal*  
17 *of Biological Sciences*, 12 (2016) 799-811. <https://doi.org/10.7150/ijbs.13310>
- 18 [18] H. Du, H. Yu, T. Ma, F. Yang, L. Jia, C. Zhang, J. Zhang, L. Niu, J. Yang, Z. Zhang, K. Zhang,  
19 Z. Li, Analysis of glycosphingolipid glycans by lectin microarrays, *Analytical Chemistry*, 91  
20 (2019) 10663-10671. <https://doi.org/10.1021/acs.analchem.9b01945>
- 21 [19] C.-C. Yeh, C.-J. Chang, Y.-C. Twu, S.-T. Hung, Y.-J. Tsai, J.-C. Liao, J.-T. Huang, Y.-H. Kao,  
22 S.-W. Lin, L.-C. Yu, The differential expression of the blood group P1-A4GALT and  
23 P2-A4GALT alleles is stimulated by the transcription factor early growth response 1,  
24 *Transfusion*, 58 (2018) 1054-1064. <https://doi.org/https://doi.org/10.1111/trf.14515>
- 25 [20] M.E. Reid, N. Mohandas, Red blood cell blood group antigens: structure and function,  
26 *Seminars in Hematology*, 41 (2004) 93-117. <https://doi.org/10.1053/j.seminhematol.2004.01.001>
- 27 [21] R.A. Flynn, K. Pedram, S.A. Malaker, P.J. Batista, B.A.H. Smith, A.G. Johnson, B.M. George,  
28 K. Majzoub, P.W. Villalta, J.E. Carette, C.R. Bertozzi, Small RNAs are modified with  
29 N-glycans and displayed on the surface of living cells, *Cell*, 184 (2021) 3109-3124.  
30 <https://doi.org/10.1016/j.cell.2021.04.023>
- 31 [22] A. Mehta, P. Norton, H. Liang, M.A. Comunale, M. Wang, L. Rodemich-Betesh, A. Koszycki,  
32 K. Noda, E. Miyoshi, T. Block, Increased levels of tetra-antennary N-linked glycan but not  
33 core fucosylation are associated with hepatocellular carcinoma tissue, *Cancer Epidemiology,*  
34 *Biomarkers & Prevention*, 21 (2012) 925-933. <https://doi.org/10.1158/1055-9965.epi-11-1183>
- 35 [23] L. Cheng, L. Cao, Y. Wu, W. Xie, J. Li, F. Guan, Z. Tan, Bisecting N-Acetylglucosamine on  
36 EGFR inhibits malignant phenotype of breast cancer via down-regulation of EGFR/Erk  
37 signaling, *Frontiers in Oncology*, 10 (2020) 929. <https://doi.org/10.3389/fonc.2020.00929>
- 38  
39  
40

- 1 [24] J. Zhang, Y. Zhong, P. Zhang, H. Du, J. Shu, X. Liu, H. Zhang, Y. Guo, Z. Jia, L. Niu, F. Yang,  
2 Z. Li, Identification of abnormal fucosylated-glycans recognized by LTL in saliva of  
3 HBV-induced chronic hepatitis, cirrhosis, and hepatocellular carcinoma, *Glycobiology*, 29  
4 (2019) 242-259. <https://doi.org/10.1093/glycob/cwy108>
- 5 [25] X. Zhou, G. Yang, F. Guan, Biological functions and analytical strategies of sialic acids in  
6 tumor, *Cells*, 9 (2020) 273. <https://doi.org/10.3390/cells9020273>
- 7 [26] C. Rajesh, P. Radhakrishnan, The (Sialyl) Tn antigen: contributions to immunosuppression in  
8 gastrointestinal cancers, *Frontiers in Oncology*, 12 (2022) 1093496.  
9 <https://doi.org/10.3389/fonc.2022.1093496>
- 10 [27] I. Brockhausen, J. Melamed, Mucins as anticancer targets: perspectives of the glycobiologist,  
11 *Glycoconjugate Journal*, 38 (2021) 459-474. <https://doi.org/10.1007/s10719-021-09986-8>
- 12 [28] C. Cumin, Y.-l. Huang, A. Everest-Dass, F. Jacob, Deciphering the importance of  
13 glycosphingolipids on cellular and molecular mechanisms associated with  
14 epithelial-to-mesenchymal transition in cancer, *Biomolecules*, 11 (2021) 62.  
15 <https://doi.org/10.3390/biom11010062>
- 16 [29] N. Berois, A. Pittini, E. Osinaga, Targeting tumor glycans for cancer therapy: successes,  
17 limitations, and perspectives, *Cancers*, 14 (2022) 645.  
18 <https://doi.org/10.3390/cancers14030645>
- 19 [30] N.K. Ibrahim, J.L. Murray, Clinical Development of the STn-KLH Vaccine (Theratope®),  
20 *Clinical Breast Cancer*, 3 (2003) S139-S143. <https://doi.org/10.3816/CBC.2003.s.003>
- 21 [31] A.M. Hernández, A.M. Vázquez, Racotumomab-alum vaccine for the treatment of  
22 non-small-cell lung cancer, *Expert Review of Vaccines* 14 (2015) 9-20.  
23 <https://doi.org/10.1586/14760584.2015.984691>
- 24 [32] C.-S. Huang, A.L. Yu, L.-M. Tseng, L.W.C. Chow, M.-F. Hou, S.A. Hurvitz, R.B. Schwab,  
25 C.-H. Wong, J.L. Murray, H.-K. Chang, H.-T. Chang, S.-C. Chen, S.-B. Kim, Y.-K. Shue, H.S.  
26 Rugo, Randomized phase II/III trial of active immunotherapy with OPT-822/OPT-821 in  
27 patients with metastatic breast cancer, *Journal of Clinical Oncology*, 34 (2016) 1003.  
28 [https://doi.org/10.1200/JCO.2016.34.15\\_suppl.1003](https://doi.org/10.1200/JCO.2016.34.15_suppl.1003)
- 29 [33] S. Dhillon, Dinutuximab: first global approval, *Drugs*, 75 (2015) 923-927. <https://doi.org/10.1007/s40265-015-0399-5>
- 30  
31 [34] Y. Gu, J. Zhang, W. Mi, J. Yang, F. Han, X. Lu, W. Yu, Silencing of GM3 synthase suppresses  
32 lung metastasis of murine breast cancer cells, *Breast Cancer Research*, 10 (2008) 1-12. <https://doi.org/10.1186/bcr1841>
- 33  
34 [35] M. Speir, I.F. Hermans, R. Weinkove, Engaging natural killer T cells as 'Universal Helpers'  
35 for vaccination, *Drugs*, 77 (2017) 1-15. <https://doi.org/10.1007/s40265-016-0675-z>
- 36 [36] Y. Makino, K. Hamamura, Y. Takei, R.H. Bhuiyan, Y. Ohkawa, Y. Ohmi, H. Nakashima, K.  
37 Furukawa, K. Furukawa, A therapeutic trial of human melanomas with combined small  
38 interfering RNAs targeting adaptor molecules p130Cas and paxillin activated under expression  
39 of ganglioside GD3, *Biochimica et biophysica acta*, 1860 (2016) 1753-1763. <https://doi.org/10.1016/j.bbagen.2016.04.005>
- 40



- 1 [37] M.H. Ravindranath, T. Tsuchida, D.L. Morton, R.F. Irie, Ganglioside GM3:GD3 ratio as an  
2 index for the management of melanoma, *Cancer*, 67 (1991) 3029-3035.  
3 [https://doi.org/10.1002/1097-0142\(19910615\)67:12<3029::aid-cnrcr2820671217>3.0.co;2-8](https://doi.org/10.1002/1097-0142(19910615)67:12<3029::aid-cnrcr2820671217>3.0.co;2-8)
- 4 [38] Y. Li, X. Huang, C. Wang, Y. Li, M. Luan, K. Ma, Ganglioside GM3 exerts opposite effects  
5 on motility via epidermal growth factor receptor and hepatocyte growth factor  
6 receptor-mediated migration signaling, *Molecular medicine reports*, 11 (2015) 2959-2966.  
7 <https://doi.org/10.3892/mmr.2014.3087>
- 8 [39] W.W. Overwijk, N.P. Restifo, B16 as a mouse model for human melanoma, *Current protocols*  
9 *in immunology*, Chapter 20 (2001). <https://doi.org/10.1002/0471142735.im2001s39>
- 10 [40] Y. Hirabayashi, A. Hamaoka, M. Matsumoto, T. Matsubara, M. Tagawa, S. Wakabayashi, M.  
11 Taniguchi, Syngeneic monoclonal antibody against melanoma antigen with interspecies  
12 cross-reactivity recognizes GM3, a prominent ganglioside of B16 melanoma, *Journal of*  
13 *Biological Chemistry*, 260 (1985) 13328-13333.  
14 [https://doi.org/10.1016/S0021-9258\(17\)38873-7](https://doi.org/10.1016/S0021-9258(17)38873-7)
- 15 [41] Y. Murozuka, N. Watanabe, K. Hatanaka, S.-i. Hakomori, Lyso-GM3, its dimer, and multimer:  
16 their synthesis, and their effect on epidermal growth factor-induced receptor tyrosine kinase,  
17 *Glycoconjugate Journal*, 24 (2007) 551-563. <https://doi.org/10.1007/s10719-007-9051-2>
- 18 [42] J.J. Lundquist, E.J. Toone, The Cluster Glycoside Effect, *Chemical Reviews*, 102 (2002)  
19 555-578. <https://doi.org/10.1021/cr000418f>
- 20 [43] G.A. Nores, T. Dohi, M. Taniguchi, S.i. Hakomori, Density-dependent recognition of cell  
21 surface GM3 by a certain anti-melanoma antibody, and GM3 lactone as a possible immunogen:  
22 requirements for tumor-associated antigen and immunogen, *Journal of Immunology*, 139 (1987)  
23 3171-3176. <https://doi.org/10.4049/jimmunol.139.9.3171>
- 24 [44] L.E. Fernandez, M.R. Gabri, M.D. Guthmann, R.E. Gomez, S. Gold, L. Fainboim, D.E.  
25 Gomez, D.F. Alonso, NGcGM3 ganglioside: a privileged target for cancer vaccines, *Clinical &*  
26 *Developmental Immunology*, 2010 (2010) 814397. <https://doi.org/10.1155/2010/814397>
- 27 [45] J.P. Oliva, Z. Valdés, A. Casacó, G. Pimentel, J. González, I. Alvarez, M. Osorio, M.P.  
28 Velasco, M. Figueroa, R. Ortiz, X. Escobar, M. Orozco, J. Cruz, S. Franco, M. Díaz, L. Roque,  
29 A. Carr, A.M. Vázquez, C. Mateos, M. Rubio, R. Pérez, L. Fernández, E. Clinical evidences of  
30 GM3 (NeuGc) ganglioside expression in human breast cancer using the 14F7 monoclonal  
31 antibody labelled with 99mTc, *Breast Cancer Research and Treatment*, 96 (2005) 115-121.  
32 <https://doi.org/10.1007/s10549-005-9064-0>
- 33 [46] A. Irie, S. Koyama, Y. Kozutsumi, T. Kawasaki, A. Suzuki, The molecular basis for the  
34 absence of N-glycolylneuraminic acid in humans, *The Journal of Biological Chemistry*, 273  
35 (1998) 15866-15871. <https://doi.org/10.1074/jbc.273.25.15866>
- 36 [47] R. Blanco, E. Domínguez, O. Morales, D. Blanco, D. Martínez, C.E. Rengifo, C. Viada, M.  
37 Cedeño, E. Rengifo, A. Carr, Prognostic significance of N-glycolyl GM3 ganglioside  
38 expression in non-small cell lung carcinoma patients: new evidences, *Pathology Research*  
39 *International*, 2015 (2015) 132326. <https://doi.org/10.1155/2015/132326>
- 40 [48] L.K. Shewell, C.J. Day, T. Hippolite, X. De Bisscop, J.C. Paton, A.W. Paton, M.P. Jennings,

- 1 Serum Neu5Gc biomarkers are elevated in primary cutaneous melanoma, *Biochemical and*  
2 *Biophysical Research Communications*, 642 (2023) 162-166. [https://doi.org/10.1016/j.bbrc.](https://doi.org/10.1016/j.bbrc.2022.12.053)  
3 [2022.12.053](https://doi.org/10.1016/j.bbrc.2022.12.053)
- 4 [49] M. Hedlund, P. Tangvoranuntakul, H. Takematsu, J.M. Long, G.D. Housley, Y. Kozutsumi, A.  
5 Suzuki, A. Wynshaw-Boris, A.F. Ryan, R.L. Gallo, N. Varki, A. Varki, N-glycolylneuraminic  
6 acid deficiency in mice: implications for human biology and evolution, *Molecular and Cellular*  
7 *Biology*, 27 (2007) 4340-4346. <https://doi.org/10.1128/mcb.00379-07>
- 8 [50] P.A. Bousquet, J.A. Sandvik, N.F. Jeppesen Edin, U. Krenzel, Hypothesis: Hypoxia induces  
9 de novo synthesis of NeuGc gangliosides in humans through CMAH domain substitute,  
10 *Biochemical and Biophysical Research Communications*, 495 (2018) 1562-1566. [https://doi.](https://doi.org/10.1016/j.bbrc.2017.11.183)  
11 [org/10.1016/j.bbrc.2017.11.183](https://doi.org/10.1016/j.bbrc.2017.11.183)
- 12 [51] A. Arcangeli, L. Toma, L. Contiero, O. Crociani, L. Legnani, C. Lunghi, E. Nesti, G. Moneti,  
13 B. Richichi, C. Nativi, Stable GM3 lactone mimetic raises antibodies specific for the antigens  
14 expressed on melanoma cells, *Bioconjugate Chemistry*, 21 (2010) 1432-1438. [https://doi.org/](https://doi.org/10.1021/bc900557v)  
15 [10.1021/bc900557v](https://doi.org/10.1021/bc900557v)
- 16 [52] A. Díaz, M. Alfonso, R. Alonso, G. Saurez, M. Troche, M. Catala, R.M. Diaz, R. Pérez, A.M.  
17 Vázquez, Immune responses in breast cancer patients immunized with an anti-idiotypic  
18 antibody mimicking NeuGc-containing gangliosides, *Clinical Immunology*, 107 (2003) 80-89.  
19 [https://doi.org/10.1016/s1521-6616\(03\)00036-6](https://doi.org/10.1016/s1521-6616(03)00036-6)
- 20 [53] H. Kaltner, S. Toegel, G.G. Caballero, J.C. Manning, R.W. Ledeen, H.-J. Gabius, Galectins:  
21 their network and roles in immunity/tumor growth control, *Histochemistry and Cell Biology*,  
22 147 (2017) 239-256. <https://doi.org/10.1007/s00418-016-1522-8>
- 23 [54] Z. Han, P.S. Thuy-Boun, W. Pfeiffer, V.F. Vartabedian, A. Torkamani, J.R. Teijaro, D.W.  
24 Wolan, Identification of an N-acetylneuraminic acid-presenting bacteria isolated from a human  
25 microbiome, *Scientific Report*, 11 (2021) 4763. <https://doi.org/10.1038/s41598-021-83875-w>
- 26 [55] P.A. M, L. B, Bacterial glycans and their interactions with lectins in the innate immune  
27 system, *Biochemical Society Transactions*, 47 (2019) 1569-1579.  
28 <https://doi.org/10.1042/bst20170410>
- 29 [56] A.R. Dochez, O.T. Avery, The elaboration of specific soluble substance by pneumococcus  
30 during growth, *The Journal of Experimental Medicine*, 26 (1917) 477. [https://doi.org/10.1084/](https://doi.org/10.1084/jem.26.4.477)  
31 [jem.26.4.477](https://doi.org/10.1084/jem.26.4.477)
- 32 [57] T. Francis Jr, W.S. Tillett, Cutaneous reactions in pneumonia. The development of antibodies  
33 following the intradermal injection of type-specific polysaccharide, *The Journal of*  
34 *Experimental Medicine*, 52 (1930) 573-585. <https://doi.org/10.1084/jem.52.4.573>
- 35 [58] M. Anderluh, F. Berti, A. Bzducha-Wróbel, F. Chiodo, C. Colombo, F. Compostella, K.  
36 Durlik, X. Ferhati, R. Holmdahl, D. Jovanovic, W. Kaca, L. Lay, M. Marinovic-Cincovic, M.  
37 Marradi, M. Ozil, L. Polito, J.J. Reina, C.A. Reis, R. Sackstein, A. Silipo, U. Švajger, O. Vaněk,  
38 F. Yamamoto, B. Richichi, S.J. van Vliet, Recent advances on smart glycoconjugate vaccines in  
39 infections and cancer, *The FEBS Journal*, 289 (2022) 4251-4303. [https://doi.org/10.1111/febs.](https://doi.org/10.1111/febs.15909)  
40 [15909](https://doi.org/10.1111/febs.15909)

- 1 [59] Z. Li, T. Bavaro, S. Tengattini, R. Bernardini, M. Mattei, F. Annunziata, R.B. Cole, C. Zheng,  
2 M. Sollogoub, L. Tamborini, M. Terreni, Y. Zhang, Chemoenzymatic synthesis of  
3 arabinomannan (AM) glycoconjugates as potential vaccines for tuberculosis, *European*  
4 *Journal of Medicinal Chemistry*, 204 (2020) 112578.  
5 <https://doi.org/10.1016/j.ejmech.2020.112578>
- 6 [60] S.S. Shivatare, V.S. Shivatare, C. Wong, Glycoconjugates: synthesis, functional studies, and  
7 therapeutic developments, *Chemical Reviews*, 122 (2022) 15603-15671.  
8 <https://doi.org/10.1021/acs.chemrev.1c01032>
- 9 [61] M.A. Cheever, J.P. Allison, A.S. Ferris, O.J. Finn, B.M. Hastings, T.T. Hecht, I. Mellman, S.A.  
10 Prindiville, J.L. Viner, L.M. Weiner, L.M. Matrisian, The prioritization of cancer antigens: a  
11 national cancer institute pilot project for the acceleration of translational research, *Clinical*  
12 *Cancer Research*, 15 (2009) 5323-5337. <https://doi.org/10.1158/1078-0432.ccr-09-0737>
- 13 [62] C.M. Snapper, J.J. Mond, A model for induction of T cell-independent humoral immunity in  
14 response to polysaccharide antigens, *Journal of Immunology*, 157 (1996) 2229-2233.  
15 <https://doi.org/10.4049/jimmunol.157.6.2229>
- 16 [63] Z. Li, C. Zheng, M. Terreni, L. Tanzi, M. Sollogoub, Y. Zhang, Novel vaccine candidates  
17 against tuberculosis, *Current Medicinal Chemistry*, 27 (2020) 5095-5118. [https://doi.org/](https://doi.org/10.2174/0929867326666181126112124)  
18 [10.2174/0929867326666181126112124](https://doi.org/10.2174/0929867326666181126112124)
- 19 [64] P. Hoyos, A. Perona, T. Bavaro, F. Berini, F. Marinelli, M. Terreni, M.J. Hernáiz,  
20 Biocatalyzed synthesis of glycostructures with anti-infective activity, *Accounts of Chemical*  
21 *Research*, 55 (2022) 2409-2424. <https://doi.org/10.1021/acs.accounts.2c00136>
- 22 [65] F. Cochet, F. Peri, The role of carbohydrates in the lipopolysaccharide (LPS)/toll-like receptor  
23 4 (TLR4) signalling, *International Journal of Molecular Sciences*, 18 (2017) 2318. [https://doi.](https://doi.org/10.3390/ijms18112318)  
24 [org/10.3390/ijms18112318](https://doi.org/10.3390/ijms18112318)
- 25 [66] F. Khatun, I. Toth, R.J. Stephenson, Immunology of carbohydrate-based vaccines, *Advanced*  
26 *Drug Delivery Reviews*, 165-166 (2020) 117-126. <https://doi.org/10.1016/j.addr.2020.04.006>
- 27 [67] Y. Baba, T. Kurosaki, Impact of Ca<sup>2+</sup> signaling on B cell function, *Trends in Immunology*, 32  
28 (2011) 589-594. <https://doi.org/10.1016/j.it.2011.09.004>
- 29 [68] A. Zandvoort, W. Timens, The dual function of the splenic marginal zone: essential for  
30 initiation of anti-TI-2 responses but also vital in the general first-line defense against  
31 blood-borne antigens, *Clinical and Experimental Immunology*, 130 (2002) 4-11.  
32 <https://doi.org/10.1046/j.1365-2249.2002.01953.x>
- 33 [69] A.H. van den Biggelaar, W.S. Pomat, Immunization of newborns with bacterial conjugate  
34 vaccines, *Vaccine*, 31 (2013) 2525-2530. <https://doi.org/10.1016/j.vaccine.2012.06.019>
- 35 [70] O.T. Avery, W.F. Goebel, Chemo-immunological studies on conjugated carbohydrate-proteins:  
36 V. The immunological specificity of an antigen prepared by combining the capsular  
37 polysaccharide of type III pneumococcus with foreign protein, *The Journal of Experimental*  
38 *Medicine*, 54 (1931) 437-447. <https://doi.org/10.1084/jem.54.3.437>
- 39 [71] F. Berti, R. Adamo, Recent mechanistic insights on glycoconjugate vaccines and future  
40 perspectives, *ACS Chemical Biology*, 8 (2013) 1653-1663. <https://doi.org/10.1021/cb400423g>

- 1 [72] F.Y. Avci, X. Li, M. Tsuji, D.L. Kasper, Carbohydrates and T cells: a sweet twosome, in:  
2 *Seminars in Immunology*, Elsevier, **2013**, pp. 146-151. [https://doi.org/10.1016/j.smim.2013.05.](https://doi.org/10.1016/j.smim.2013.05.005)  
3 [005](https://doi.org/10.1016/j.smim.2013.05.005)
- 4 [73] C. Sorieul, F. Papi, F. Carboni, S. Pecetta, S. Phogat, R. Adamo, Recent advances and future  
5 perspectives on carbohydrate-based cancer vaccines and therapeutics, *Pharmacology &*  
6 *Therapeutics*, 235 (2022) 108158. <https://doi.org/10.1016/j.pharmthera.2022.108158>
- 7 [74] L. CC, Y. XS, Carbohydrate-based cancer vaccines: target cancer with sugar bullets,  
8 *Glycoconjugate Journal*, 29 (2012) 259-271. <https://doi.org/10.1007/s10719-012-9399-9>
- 9 [75] F. Diniz, P. Coelho, H.O. Duarte, B. Sarmiento, C.A. Reis, J. Gomes, Glycans as targets for  
10 drug delivery in cancer, *Cancers*, 14 (2022) 911. <https://doi.org/10.3390/cancers14040911>
- 11 [76] N. Pishesha, T.J. Harmand, H.L. Ploegh, A guide to antigen processing and presentation,  
12 *Nature Reviews Immunology*, 22 (2022) 751-764. <https://doi.org/10.1038/s41577-022-00707-2>
- 13 [77] S.A. Malaker, M.J. Ferracane, F.R. Depontieu, A.L. Zarling, J. Shabanowitz, D.L. Bai, S.L.  
14 Topalian, V.H. Engelhard, D.F. Hunt, Identification and characterization of complex  
15 glycosylated peptides presented by the MHC class II processing pathway in melanoma,  
16 *Journal of Proteome Research*, 16 (2017) 228-237.  
17 <https://doi.org/10.1021/acs.jproteome.6b00496>
- 18 [78] M.A. Wolfert, G.-J. Boons, Adaptive immune activation: glycosylation does matter, *Nature*  
19 *Chemical Biology*, 9 (2013) 776-784. <https://doi.org/10.1038/nchembio.1403>
- 20 [79] A. Glithero, J. Tormo, J.S. Haurum, G. Arsequell, G. Valencia, J. Edwards, S. Springer, A.  
21 Townsend, Y.-L. Pao, M. Wormald, Crystal structures of two H-2Db/glycopeptide complexes  
22 suggest a molecular basis for CTL cross-reactivity, *Immunity*, 10 (1999) 63-74. [https://doi.org/](https://doi.org/10.1016/S1074-7613(00)80007-2)  
23 [10.1016/S1074-7613\(00\)80007-2](https://doi.org/10.1016/S1074-7613(00)80007-2)
- 24 [80] A. Vasso, Y. Elizabeth, A.R. Paul, H. Jodie, O. Carla, L. Wenjun, P. Magdalena, P. Hans,  
25 F.C.M. Ian, A glycopeptide in complex with MHC class I uses the GalNAc residue as an  
26 anchor, *Proceedings of the National Academy of Sciences (PNAS)*, 100 (2003) 15029-15034.  
27 <https://doi.org/10.1073/pnas.2432220100>
- 28 [81] J.A. Speir, U.M. Abdel-Motal, M. Jondal, I.A. Wilson, Crystal structure of an MHC class I  
29 presented glycopeptide that generates carbohydrate-specific CTL, *Immunity*, 10 (1999) 51-61.  
30 [https://doi.org/10.1016/s1074-7613\(00\)80006-0](https://doi.org/10.1016/s1074-7613(00)80006-0)
- 31 [82] G.-H. Wilton, E.P. Tenorio, F.R.C. Sanchez, M.C. Mendoza, R.L. Ledezma, E. Zenteno,  
32 Relevance of glycans in the interaction between T lymphocyte and the antigen presenting cell,  
33 *International Reviews of Immunology*, 40 (2021) 274-288. [https://doi.org/10.1080/08830185.](https://doi.org/10.1080/08830185.2020.1845331)  
34 [2020.1845331](https://doi.org/10.1080/08830185.2020.1845331)
- 35 [83] M. Basler, C.J. Kirk, M. Groettrup, The immunoproteasome in antigen processing and other  
36 immunological functions, *Current Opinion in Immunology*, 25 (2013) 74-80.  
37 <https://doi.org/10.1016/j.coi.2012.11.004>
- 38 [84] M.A. Garstka, J. Neefjes, How to target MHC class II into the MIIC compartment, *Molecular*  
39 *Immunology*, 55 (2013) 162-165. <https://doi.org/10.1016/j.molimm.2012.10.022>
- 40 [85] Y. Wu, N. Zhang, K. Hashimoto, C. Xia, J.M. Dijkstra, Structural comparison between MHC

- 1 classes I and II; in evolution, a class-II-like molecule probably came first, *Frontiers in*  
2 *Immunology*, 12 (2021) 621153. <https://doi.org/10.3389/fimmu.2021.621153>
- 3 [86] M. Cavallari, P. Stallforth, A. Kalinichenko, D.C. Rathwell, T.M. Gronewold, A. Adibekian, L.  
4 Mori, R. Landmann, P.H. Seeberger, G. De Libero, A semisynthetic carbohydrate-lipid vaccine  
5 that protects against *S. pneumoniae* in mice, *Nature Chemical Biology*, 10 (2014) 950-956.  
6 <https://doi.org/10.1038/nchembio.1650>
- 7 [87] Y. Zhang, R. Springfield, S. Chen, X. Li, X. Feng, R. Moshirian, R. Yang, W. Yuan,  $\alpha$ -GalCer  
8 and iNKT cell-based cancer immunotherapy: realizing the therapeutic potentials, *Frontiers in*  
9 *Immunology*, 10 (2019) 1126. <https://doi.org/10.3389/fimmu.2019.01126>
- 10 [88] S. Kim, S. Lalani, V.V. Parekh, L. Wu, L. Van Kaer, Glycolipid ligands of invariant natural  
11 killer T cells as vaccine adjuvants, *Expert Review of Vaccines*, 7 (2008) 1519-1532. [https://](https://doi.org/10.1586/14760584.7.10.1519)  
12 [doi.org/10.1586/14760584.7.10.1519](https://doi.org/10.1586/14760584.7.10.1519)
- 13 [89] L.J. Carreño, N.A. Saavedra-Ávila, S.A. Porcelli, Synthetic glycolipid activators of natural  
14 killer T cells as immunotherapeutic agents, *Clinical & Translational Immunology*, 5 (2016) e69.  
15 <https://doi.org/10.1038/cti.2016.14>
- 16 [90] Z. Huang, Z. Sun, Y. Gao, P. Chen, Y. Liu, Y. Chen, Y. Li, Strategy for designing a synthetic  
17 tumor vaccine: multi-component, multivalency and antigen modification, *Vaccines*, 2(2014)  
18 549-562. <https://doi.org/10.3390/vaccines2030549>
- 19 [91] D. Adi, R. Govindaswami, S. Wolfgang, K. Panageas, C. Hudis, P.O. Livingston, G. Teresa, A  
20 pilot study of vaccination with sialyl Lewis x (sLe<sup>x</sup>)–keyhole limpet hemocyanin (KLH)  
21 conjugate plus the immunologic adjuvant QS-21 in metastatic breast cancer patients (pts),  
22 *Journal of Clinical Oncology*, (2011). [https://doi.org/10.1200/jco.2011.29.15](https://doi.org/10.1200/jco.2011.29.15_suppl.2599) suppl.2599
- 23 [92] E. Kagan, G. Ragupathi, S. Yi, C.A. Reis, J. Gildersleeve, D. Kahne, H. Clausen, S.J.  
24 Danishefsky, P.O. Livingston, Comparison of antigen constructs and carrier molecules for  
25 augmenting the immunogenicity of the monosaccharide epithelial cancer antigen Tn, *Cancer*  
26 *Immunology, Immunotherapy*, 54 (2005) 424-430. <https://doi.org/10.1007/s00262-004-0584-y>
- 27 [93] R.A. Peter, Developments in CRM197 glycoconjugates for anticancer vaccines, *American*  
28 *Journal of Biomedical Science & Research*, 6 (2019) 212-215. [https://doi.org/10.34297/ajbsr.](https://doi.org/10.34297/ajbsr.2019.06.001031)  
29 [2019.06.001031](https://doi.org/10.34297/ajbsr.2019.06.001031)
- 30 [94] R. Curtiss, Vaccine design: innovative approaches and novel strategies, *Expert Review of*  
31 *Vaccines*, 10 (2011) 1385-1387. <https://doi.org/10.1586/erv.11.114>
- 32 [95] B. Sarkar, N. Jayaraman, Glycoconjugations of biomolecules by chemical methods, *Frontiers*  
33 *in Chemistry*, 8 (2020). <https://doi.org/10.3389/fchem.2020.570185>
- 34 [96] A.B. Michael, D.H. Laurance, A general synthesis of model glycoproteins: coupling of  
35 alkenyl glycosides to proteins, using reductive ozonolysis followed by reductive amination  
36 with sodium cyanoborohydride, *Carbohydrate Research*, 78 (1980) C1-C3.  
37 [https://doi.org/10.1016/s0008-6215\(00\)83676-9](https://doi.org/10.1016/s0008-6215(00)83676-9)
- 38 [97] X. Zheng, F. Yang, M. Zheng, C. Huo, Y. Zhang, X. Ye, Improvement of the immune efficacy  
39 of carbohydrate vaccines by chemical modification on the GM3 antigen, *Organic &*  
40 *Biomolecular Chemistry*, 13 (2015) 6399-6406. <https://doi.org/10.1039/c5ob00405e>



- 1 [98] J. Xue, Y. Pan, Z. Guo, Neoglycoprotein cancer vaccines: Synthesis of an azido derivative of  
2 GM3 and its efficient coupling to proteins through a new linker, *Tetrahedron Letters*, 43 (2002)  
3 1599-1602. [https://doi.org/10.1016/S0040-4039\(02\)00071-0](https://doi.org/10.1016/S0040-4039(02)00071-0)
- 4 [99] Y. Pan, P. Chefalo, N. Nagy, C. Harding, Z. Guo, Synthesis and immunological properties of  
5 N-modified GM3 antigens as therapeutic cancer vaccines, *Journal of Medicinal Chemistry*, 48  
6 (2005) 875-883. <https://doi.org/10.1021/jm0494422>
- 7 [100] R. Roy, L. Mousavifar, Carrier diversity and chemical ligations in the toolbox for designing  
8 tumor-associated carbohydrate antigens (TACAs) as synthetic vaccine candidates, *Chemical*  
9 *Society Reviews*, 52 (2023) 3353-3396. <https://doi.org/10.1039/D2CS01032A>
- 10 [101] O.V. Kozyreva, E.M. Novikova, E.A. Khatuntseva, Y.E. Tsvetkov, N.E. Nifantiev, R.N.  
11 Stepanenko, Antitumor effect of the conjugate of synthetic carbohydrate moiety of the  
12 tumor-associated ganglioside GM3 with hemocyanin in combination with the cytostatic  
13 cyclophosphamide, *Russian Chemical Bulletin*, 63 (2014) 1431-1437. [https://doi.org/10.1007/](https://doi.org/10.1007/s11172-014-0614-7)  
14 [s11172-014-0614-7](https://doi.org/10.1007/s11172-014-0614-7)
- 15 [102] E.M. Novikova, E.A. Khatuntseva, Y.E. Tsvetkov, N.A. Razvalyaeva, D.A. Goncharuk, O.A.  
16 Zeynalov, N.E. Nifantiev, R.N. Stepanenko, Synthesis of a conjugate of 3'-sialyllactoside with  
17 recombinant flagellin as a carrier protein and assessment of its immunological activity in  
18 comparison with that of a similar hemocyanin-based conjugate, *Russian Chemical Bulletin*, 64  
19 (2015) 1640-1647. <https://doi.org/10.1007/s11172-015-1054-8>
- 20 [103] X. Yin, J. Lu, J. Wang, R. Zhang, X. Wang, C. Liao, X. Liu, Z. Liu, J. Guo, Synthesis and  
21 evaluation of liposomal anti-GM3 cancer vaccine candidates covalently and noncovalently  
22 adjuvanted by  $\alpha$ GalCer, *Journal of Medicinal Chemistry*, 64 (2021) 1951-1965. [https://doi.org/](https://doi.org/10.1021/acs.jmedchem.0c01186)  
23 [10.1021/acs.jmedchem.0c01186](https://doi.org/10.1021/acs.jmedchem.0c01186)
- 24 [104] C. Romanò, H. Jiang, S. Tahvili, P. Wei, U.B. Keiding, G. Clergeaud, J.R. Henriksen, T.L.  
25 Andresen, A.E. Hansen, D. Christensen, M. Clausen, Hartvig, Chemical synthesis and  
26 immunological evaluation of cancer vaccines based on ganglioside antigens and  
27  $\alpha$ -galactosylceramide, *ChemRxiv*, 30 July (2021). <https://doi.org/10.26434/chemrxiv-2021-12sv>
- 28 [105] J.C. Manimala, T.A. Roach, Z. Li, J.C. Gildersleeve, High-throughput carbohydrate  
29 microarray profiling of 27 antibodies demonstrates widespread specificity problems,  
30 *Glycobiology*, 17 (2007) 17-23. <https://doi.org/10.1093/glycob/cwm047>
- 31 [106] A.V. Casadesús, Y. Fernandez-Marrero, M. Clavell, J.A. Gómez, T. Hernández, E. Moreno,  
32 A. López-Requena, A shift from N-glycolyl- to N-acetyl-sialic acid in the GM3 ganglioside  
33 impairs tumor development in mouse lymphocytic leukemia cells, *Glycoconjugate Journal*, 30  
34 (2013) 687-699. <https://doi.org/10.1007/s10719-013-9473-y>
- 35 [107] J. Grabowska, D.A. Stolk, M.K.N. Twilhaar, M. Ambrosini, G. Storm, H.J. van der Vliet,  
36 T.D. de Gruijl, Y. van Kooyk, J.M.M. den Haan, Liposomal nanovaccine containing  
37  $\alpha$ -galactosylceramide and ganglioside GM3 stimulates robust CD8+ T Cell responses via  
38 CD169+ macrophages and cDC1, *Vaccines*, 9 (2021) 56.  
39 <https://doi.org/10.3390/vaccines9010056>
- 40 [108] M.K. Nijen Twilhaar, L. Czentner, R.G. Bouma, K. Olesek, J. Grabowska, A.Z. Wang, A.J.

- 1 Affandi, S.C. Belt, H. Kalay, C.F. van Nostrum, Y. van Kooyk, G. Storm, J.M.M. den Haan,  
2 Incorporation of toll-like receptor ligands and inflammasome stimuli in GM3 liposomes to  
3 induce dendritic cell maturation and T cell responses, *Frontiers in Immunology*, (2022).  
4 <https://doi.org/10.3389/fimmu.2022.842241>
- 5 [109] D.G. Cox, L. Fox, R. Tian, W. Bardet, M. Skaley, D. Mojsilovic, J.E. Gumperz, W.H.  
6 Hildebrand, Determination of cellular lipids bound to human CD1d molecules, *Plos One*, 4  
7 (2009) e5325. <https://doi.org/10.1371/journal.pone.0005325>
- 8 [110] K. Muindi, M. Cernadas, G.F. Watts, L. Royle, D.C.A. Neville, R.A. Dwek, G.S. Besra, P.M.  
9 Rudd, T.D. Butters, M.B. Brenner, Activation state and intracellular trafficking contribute to  
10 the repertoire of endogenous glycosphingolipids presented by CD1d, *Proceedings of the*  
11 *National Academy of Sciences (PNAS)*, 107 (2010) 3052-3057.  
12 <https://doi.org/10.1073/pnas.0915056107>
- 13 [111] M.V. Gentilini, M.J.L. Pérez, P. Fernandez, L. Fainboim, E. Arana, The tumor antigen  
14 N-glycolyl-GM3 is a human CD1d ligand capable of mediating B cell and natural killer T cell  
15 interaction, *Cancer Immunology, Immunotherapy*, 65 (2016) 551-562. <https://doi.org/10.1007/s00262-016-1812-y>
- 16  
17 [112] C.R. Casella, T.C. Mitchell, Putting endotoxin to work for us: Monophosphoryl lipid A as a  
18 safe and effective vaccine adjuvant, *Cellular and Molecular Life Sciences*, 65 (2008)  
19 3231-3240. <https://doi.org/10.1007/s00018-008-8228-6>
- 20 [113] C.L. Ruegg, R.I. Jaffe, B. Köster, J.C. Sadoff, W.D. Zolliger, G.H. Lowell, M. Strand,  
21 Preparation of proteosome-based vaccines correlation of immunogenicity with physical  
22 characteristics, *Journal of Immunological Methods*, 135 (1990) 101-109. [https://doi.org/10.1016/0022-1759\(90\)90262-T](https://doi.org/10.1016/0022-1759(90)90262-T)
- 23  
24 [114] A.D. F, G.M. R, G.M. D, F. Leonardo, G.D. E, A novel hydrophobized GM3  
25 ganglioside/Neisseria meningitidis outer-membrane-protein complex vaccine induces tumor  
26 protection in B16 murine melanoma, *International Journal of Oncology*, 15 (1999) 59-66.  
27 <https://doi.org/10.3892/ijco.15.1.59>
- 28 [115] A. Carr, Z. Mazorra, D.F. Alonso, C. Mesa, O. Valiente, D.E. Gomez, R. Perez, L.E.  
29 Fernandez, A purified GM3 ganglioside conjugated vaccine induces specific,  
30 adjuvant-dependent and non-transient antitumour activity against B16 mouse melanoma in  
31 vitro and in vivo, *Melanoma Research*, 11 (2001).  
32 <https://doi.org/10.1097/00008390-200106000-00003>
- 33 [116] C. Venier, M.D. Guthmann, L.E. Fernández, L. Fainboim, Innate-immunity cytokines  
34 induced by very small size proteoliposomes, a Neisseria-derived immunological adjuvant,  
35 *Clinical and Experimental Immunology*, 147 (2007) 379-388.  
36 <https://doi.org/10.1111/j.1365-2249.2006.03297.x>
- 37 [117] M.D. Guthmann, R.J. Bitton, A.J.L. Carnero, M.R. Gabri, G. Cinat, L. Koliren, D. Lewi, L.E.  
38 Fernández, D.F. Alonso, D.E. Gomez, L. Fainboim, Active specific immunotherapy of  
39 melanoma with a GM3 ganglioside-based vaccine, *Journal of Immunotherapy*, 27 (2004)  
40 442-451. <https://doi.org/10.1097/00002371-200411000-00004>

- 1 [118] C. Erridge, E. Bennett-Guerrero, I.R. Poxton, Structure and function of lipopolysaccharides,  
2 *Microbes and Infection*, 4 (2002) 837-851. [https://doi.org/10.1016/s1286-4579\(02\)01604-0](https://doi.org/10.1016/s1286-4579(02)01604-0)
- 3 [119] A. Shimoyama, K. Fukase, Chemical synthesis and immunomodulatory functions of  
4 bacterial lipid A, *Methods in Molecular Biology*, 2613 (2023) 33-53.  
5 [https://doi.org/10.1007/978-1-0716-2910-9\\_4](https://doi.org/10.1007/978-1-0716-2910-9_4)
- 6 [120] Y. Zhang, J. Gaekwad, M. Wolfert, A. G.-J. Boons, Modulation of innate immune responses  
7 with synthetic lipid A derivatives, *Journal of the American Chemical Society*, 129 (2007)  
8 5200-5216. <https://doi.org/10.1021/ja068922a>
- 9 [121] S. Tang, Q. Wang, Z. Guo, Synthesis of a monophosphoryl derivative of Escherichia coli  
10 lipid A and its efficient coupling to a tumor-associated carbohydrate antigen, *Chemistry*, 16  
11 (2010) 1319-1325. <https://doi.org/10.1002/chem.200902153>
- 12 [122] Q. Wang, Z. Zhou, S. Tang, Z. Guo, Carbohydrate-monophosphoryl lipid A conjugates are  
13 fully synthetic self-adjuvanting cancer vaccines eliciting robust immune responses in the  
14 mouse, *ACS Chemical Biology*, 7 (2011) 235-240. <https://doi.org/10.1021/cb200358>
- 15 [123] M.K. Nijen Twilhaar, L. Czentner, J. Grabowska, A.J. Affandi, C.Y.J. Lau, K. Olesek, H.  
16 Kalay, C.F. Van Nostrum, Y. Van Kooyk, G. Storm, Optimization of liposomes for antigen  
17 targeting to splenic CD169+ macrophages, *Pharmaceutics*, 12 (2020) 1138. <https://doi.org/10.3390/pharmaceutics12121138>
- 18 [124] S. Shaik, R. Kumar, M. Chaudhary, C. Kaur, N. Khurana, G. Singh, Artificial viruses: a  
19 nanotechnology based approach, *DARU Journal of Pharmaceutical Sciences*, (2023). <https://doi.org/10.1007/s40199-023-00496-6>
- 20 [125] A. Miermont, H. Barnhill, E. Strable, X. Lu, K.A. Wall, Q. Wang, M.G. Finn, X. Huang,  
21 Cowpea mosaic virus capsid: a promising carrier for the development of carbohydrate based  
22 antitumor vaccines, *Chemistry*, 14 (2008) 4939-4947. <https://doi.org/10.1002/chem.200800203>
- 23 [126] Z. Rashidjahanabad, S. Ramadan, N.A. O'Brien, A. Nakisa, S. Lang, H. Crawford, J.C.  
24 Gildersleeve, X. Huang, Stereoselective synthesis of sialyl lewis(a) antigen and the effective  
25 anticancer activity of its bacteriophage Q $\beta$  conjugate as an anticancer vaccine, *Angewandte*  
26 *Chemie*, 62 (2023) e202309744. <https://doi.org/10.1002/anie.202309744>
- 27 [127] G. Gunay, M. Sardan Ekiz, X. Ferhati, B. Richichi, C. Nativi, A.B. Tekinay, M.O. Guler,  
28 Antigenic GM3 lactone mimetic molecule integrated mannosylated glycopeptide nanofibers  
29 for the activation and maturation of dendritic cells, *ACS Applied Materials & Interfaces*, 9  
30 (2017) 16035-16042. <https://doi.org/10.1021/acsami.7b04094>
- 31 [128] R. Ribeiro-Viana, E. Bonechi, J. Rojo, C. Ballerini, G. Comito, B. Richichi, C. Nativi,  
32 Human dendritic cell activation induced by a permannosylated dendron containing an antigenic  
33 GM3-lactone mimetic, *Beilstein Journal of Organic Chemistry*, 10 (2014) 1317-1324.  
34 <https://doi.org/10.3762/bjoc.10.133>
- 35 [129] P. Arosio, G. Comito, F. Orsini, A. Lascialfari, P. Chiarugi, C. Ménard-Moyon, C. Nativi, B.  
36 Richichi, Conjugation of a GM3 lactone mimetic on carbon nanotubes enhances the related  
37 inhibition of melanoma-associated metastatic events, *Organic & Biomolecular Chemistry*, 16  
38 (2018) 6086-6095. <https://doi.org/10.1039/c8ob01817k>
- 39  
40



- 1 [130] Z.R. Laughrey, S.E. Kiehna, A.J. Riemen, M.L. Waters, Carbohydrate- $\pi$  interactions: what  
2 are they worth?, *Journal of the American Chemical Society*, 130 (2008) 14625-14633.  
3 <https://doi.org/10.1021/ja803960x>
- 4 [131] O. Haji-Ghassemi, R.J. Blackler, M.N. Young, S.V. Evans, Antibody recognition of  
5 carbohydrate epitopes, *Glycobiology*, 25 (2015) 920-952. [https://doi.org/10.1093/glycob/  
6 cwv037](https://doi.org/10.1093/glycob/cwv037)
- 7 [132] V.I. Segatori, G.M. Ferreira, S. Rojo, A.C. Nogueira, J.O. Castillo, C.A. Gulino, M.R. Gabri,  
8 Mimicry of Tumour-Associated Carbohydrates: Is It a Promising Option for Cancer  
9 Treatment?, *Immuno*, 3 (2023) 122-147. <https://doi.org/10.3390/immuno3020009>
- 10 [133] T. Dohi, G. Nores, S.-I. Hakomori, An IgG3 monoclonal antibody established after  
11 immunization with GM3 lactone: immunochemical specificity and inhibition of melanoma cell  
12 growth in vitro and in vivo, *Cancer research*, 48 (1988) 5680-5685.
- 13 [134] K. Ding, A. Rosén, A.K. Ray, G. Magnusson, Anti-GM3-lactam monoclonal antibodies of  
14 the IgG type recognize natural GM3-ganglioside lactone but not GM3-ganglioside,  
15 *Glycoconjugate Journal*, 9 (1992) 303-306. <https://doi.org/10.1007/bf00731090>
- 16 [135] L. Toma, E. Di Cola, A. Ienco, L. Legnani, C. Lunghi, G. Moneti, B. Richichi, S. Ristori, D.  
17 Dell'Atti, C. Nativi, Synthesis, conformational studies, binding assessment and liposome  
18 insertion of a thioether- bridged mimetic of the antigen GM3 ganglioside lactone,  
19 *ChemBioChem*, 8 (2007) 1646-1649. <https://doi.org/10.1002/cbic.200700208>
- 20 [136] G. Hirai, M. Kato, H. Koshino, E. Nishizawa, K. Oonuma, E. Ota, T. Watanabe, D.  
21 Hashizume, Y. Tamura, M. Okada, T. Miyagi, M. Sodeoka, Ganglioside GM3 analogues  
22 containing monofluoromethylene-linked sialoside: synthesis, stereochemical effects,  
23 conformational behavior, and biological activities, *Journal of the American Chemical Society*,  
24 1 (2021) 137-146. <https://doi.org/10.1021/jacsau.0c00058>
- 25 [137] J.R. Rich, D.R. Bundle, S-linked ganglioside analogues for use in conjugate vaccines,  
26 *Organic Letters*, 6 (2004) 897-900. <https://doi.org/10.1021/ol036460p>
- 27 [138] J.R. Rich, W.W. Wakarchuk, D.R. Bundle, Chemical and chemoenzymatic synthesis of  
28 S-linked ganglioside analogues and their protein conjugates for use as immunogens, *Chemistry*,  
29 12 (2006) 845-858. <https://doi.org/10.1002/chem.200500518>
- 30 [139] D.R. Bundle, J.R. Rich, S. Jacques, H.N. Yu, M. Nitz, C. Ling, Thiooligosaccharide  
31 conjugate vaccines evoke antibodies specific for native antigens, *Angewandte Chemie*, 44  
32 (2005) 7725-7729. <https://doi.org/10.1002/anie.200502179>
- 33 [140] G. Hirai, Sialidase-resistant ganglioside GM3 analogues: evaluation of biological activity,  
34 *Methods in Molecular Biology*, 2613 (2023) 79-87.  
35 [https://doi.org/10.1007/978-1-0716-2910-9\\_7](https://doi.org/10.1007/978-1-0716-2910-9_7)
- 36 [141] F. Yang, X.-J. Zheng, C.-X. Huo, Y. Wang, Y. Zhang, X.-S. Ye, Enhancement of the  
37 immunogenicity of synthetic carbohydrate vaccines by chemical modifications of STn antigen,  
38 *ACS Chemical Biology*, 6 (2011) 252-259. <https://doi.org/10.1021/cb100287q>
- 39 [142] J. Wang, D. Lu, R. Sun, S. Lei, S. Luo, X. Dang, Y. Zhang, C. Yuan, Y. Zhang, J. Wu, G.  
40 Yang, L. Fu, F. Jiang, One-pot enzymatic synthesis and biological evaluation of ganglioside

- 1 GM3 derivatives as potential cancer immunotherapeutics, *Journal of Medicinal Chemistry*, 65  
2 (2022) 1883-1897. <https://doi.org/10.1021/acs.jmedchem.1c01301>
- 3 [143] J. Du, M.A. Meledeo, Z. Wang, H.S. Khanna, V.D.P. Paruchuri, K.J. Yarema, Metabolic  
4 glycoengineering: sialic acid and beyond, *Glycobiology*, 19 (2009) 1382-1401. [https://doi.org/](https://doi.org/10.1093/glycob/cwp115)  
5 [10.1093/glycob/cwp115](https://doi.org/10.1093/glycob/cwp115)
- 6 [144] J.A. Hanover, Glycan-dependent signaling: O-linked N-acetylglucosamine, *The FASEB*  
7 *Journal*, 15 (2001) 1865-1876. <https://doi.org/10.1096/fj.01-0094rev>
- 8 [145] S. Carolin, P. Stehling, J. Schnitzer, W. Reutter, R.d. Horstkorte, Biochemical engineering of  
9 neural cell surfaces by the synthetic N-propanoyl-substituted neuraminic acid precursor,  
10 *Journal of Biological Chemistry*, 273 (1998) 19146-19152.  
11 <https://doi.org/10.1074/jbc.273.30.19146>
- 12 [146] M. Boyce, S.A. Malaker, N.M. Riley, J.J. Kohler, The 2022 Nobel Prize in chemistry-sweet!,  
13 *Glycobiology*, 33 (2023) 178-181. <https://doi.org/10.1093/glycob/cwad016>
- 14 [147] P. Chefalo, Y. Pan, N. Nagy, Z. Guo, C.V. Harding, Efficient metabolic engineering of GM3  
15 on tumor cells by N-phenylacetyl-D-mannosamine, *Biochemistry*, 45 (2006) 3733-3739.  
16 <https://doi.org/10.1021/bi052161r>
- 17 [148] L. Qiu, J. Li, S. Yu, Q. Wang, Y. Li, Z. Hu, Q. Wu, Z. Guo, J. Zhang, A novel cancer  
18 immunotherapy based on the combination of a synthetic carbohydrate-pulsed dendritic cell  
19 vaccine and glycoengineered cancer cells, *Oncotarget*, 6 (2015) 5195-5203. [https://doi.org/](https://doi.org/10.18632/oncotarget.2908)  
20 [10.18632/oncotarget.2908](https://doi.org/10.18632/oncotarget.2908)
- 21 [149] H. Lin, H. Hong, L. Feng, J. Shi, Z. Zhou, Z. Wu, Synthesis of DNP-modified GM3-based  
22 anticancer vaccine and evaluation of its immunological activities for cancer immunotherapy,  
23 *Chinese Chemical Letters*, 32 (2021) 4041-4044.  
24 <https://doi.org/doi.org/10.1016/j.cclet.2021.04.034>