ACCOUNTS of chemical research

Article

pubs.acs.org/accounts

Development of Globo-H Cancer Vaccine

Published as part of the Accounts of Chemical Research special issue "Synthesis, Design, and Molecular Function".

Samuel J. Danishefsky,*[,†] Youe-Kong Shue,[‡] Michael N. Chang,[‡] and Chi-Huey Wong*[,§,⊥]

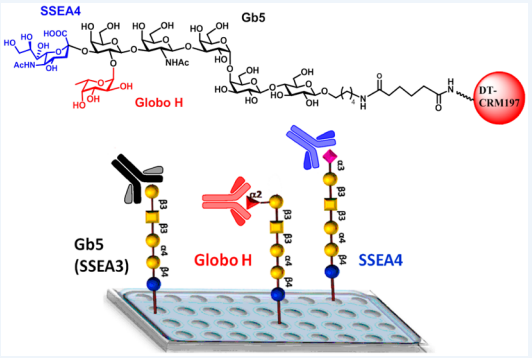
[†]Memorial Sloan Kettering Cancer Center, Sloan Kettering Institute, 1275 York Avenue, New York, New York 10065, United States

[‡]OBI Pharma, Inc., 19th F, 3 Yuan Qu Street, Taipei 11503, Taiwan

[§]The Genomics Research Center, Academia Sinica, 128 Academia Road, Sec. 2, Taipei, Taiwan

[⊥]The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, United States

CONSPECTUS: The development of anticancer vaccines requires the identification of unique epitope markers, preferably expressed exclusively on the surface of cancer cells. This Account describes the path of development of a carbohydrate-based vaccine for metastatic breast cancer, including the selection and synthesis of Globo-H as the target, the development of the vaccine conjugate and adjuvant design, the study of the immune response and consideration of class switch, and the analysis of Globo-H distribution on the surface of various cancer cells, cancer stem cells, and normal cells. The first synthesis of Globo-H was accomplished through the use of glycal chemistry; this approach delivered sufficient material for evaluation in phase I human trials. The development of a programmable one-pot synthesis method rendered the synthesis more practical and enabled the midstage proof-of-concept phase II trial and late-stage phase III trial. Finally, enzymatic synthesis of Globo-H coupled with cofactor regeneration was used for the late-stage multicenter trials and manufacture of the product. Along this path of development, it was discovered that the vaccine induced antibodies to target not only Globo-H, but also SSEA3 and SSEA4. Moreover, these three glycolipids were found to be uniquely expressed not only on the cell surface of breast cancer but on 15 additional cancer types, suggesting the broad application of this vaccine in cancer treatment and perhaps cancer prevention. In addition, a new glycolipid adjuvant was designed to target the CD1d receptor on dendritic cells and B cells for presentation to and activation of T cells to modulate the immune response and induce a class switch from IgM to IgG, thereby overcoming the common problem of carbohydrate-based vaccines that often induce mainly IgM antibodies.



As demonstrated in this vaccine development, the chemical approach to the synthesis and conjugation of carbohydrate-based immunogens provides the flexibility for access to various structures and linkers to identify optimal compositions for development. The enzymatic method was then introduced to enable the practical synthesis of the vaccine candidate for clinical development and commercialization. Overall, this Account illustrates the path of development of a cancer vaccine, from selection of a unique glycan marker on breast cancer cells and the cancer stem cells as target to the use of chemistry in combination with immunology and cancer biology to enable the design and development of the Globo-H vaccine to target three specific glycan markers exclusively expressed on the cell surface of a number of different types of cancer.

INTRODUCTION

A fundamental objective of tumor immunology is to harness the power of the human immune system in the battle against cancer. Progress toward this goal requires the careful design of vaccine candidates that reflect phenotypic distinctions between normal and malignant cells. Along these lines, it is of note that cancer cells may be differentiated from normal cells by the presentation on their surfaces of unusual glycosylation patterns: tumor-associated carbohydrate antigens (TACAs) may be expressed as membrane-bound glycoproteins or glycolipids.[1] Researchers have long been intrigued by the possibility of designing TACA-based anticancer vaccine constructs that, when properly presented to the immune system, might induce a robust antibody response, leading to the selective eradication of TACA-presenting tumor cells. In support of this general concept are reports that, in rare instances, cancer patients are able to raise natural TACA-directed antibody responses; intriguingly, survival rates are significantly improved in patients that exhibit a natural immune response to these tumor-associated carbohydrate antigens.[2] Examples include the glycopeptide vaccines targeting MUC1 and carbohydrate vaccines targeting GM2 and Globo-H.[1,2] However, to ensure efficacy and avoid the problem of autoimmunity, the TACA selected for vaccine design must be uniquely expressed on the surface of cancer cells but not on normal cells. This requirement

Received: November 20, 2014
Published: February 10, 2015

DOI: 10.1021/ar5004187
Acc. Chem. Res. 2015, 48, 643−652

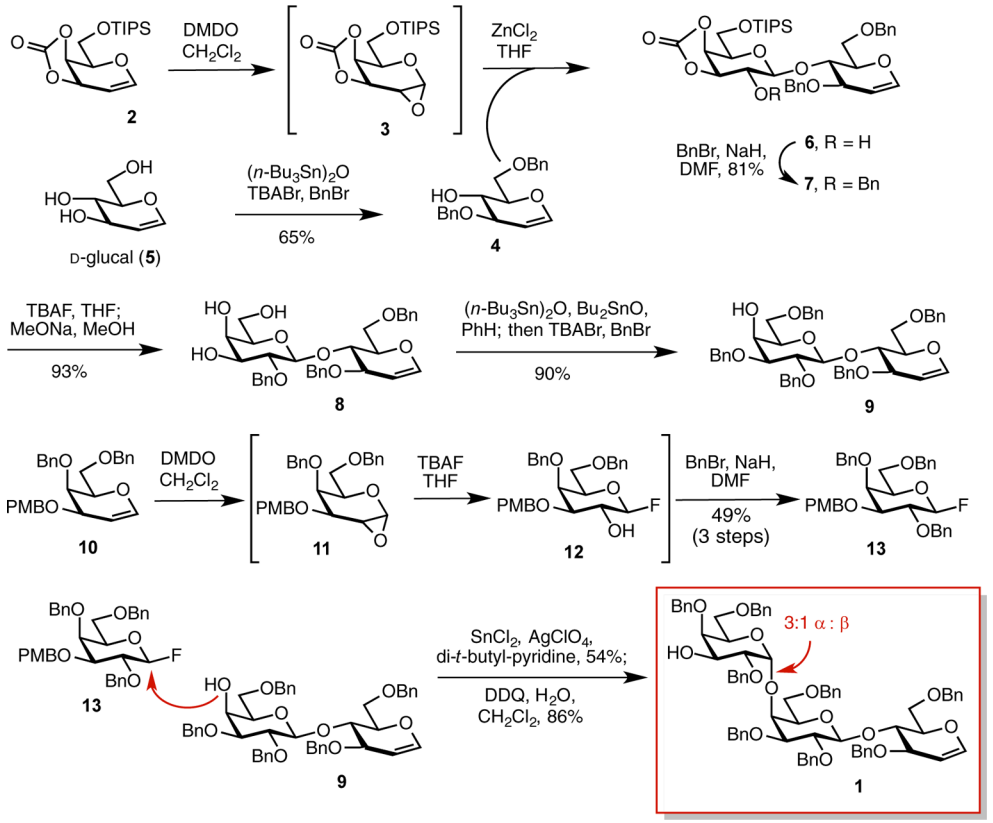


Figure 1. Synthesis of the Globo-H glycal: ABC trisaccharide.

is very challenging because most TACAs are also found on normal cells, though they are more highly expressed on cancer cells.

For over 25 years, the group at Memorial Sloan Kettering Cancer Center (MSKCC) has been engaged in a program directed toward the synthesis and evaluation of carbohydrate-based anticancer vaccine candidates. Our original involvement in this area arose from an interest in accomplishing the chemical synthesis of polysaccharides through glycal assembly methods developed in our laboratory. Fruitful discussions with Phil Livingston and Lloyd Old, pioneers in the field of TACA-based immunology, inspired us to undertake the syntheses of a class of tumor-associated blood group antigens, whose members include LewisY and LewisX. Indeed, these polysaccharides were prepared in our laboratory in homogeneous form through application of our glycal assembly technology. These synthetic TACAs were appended to the immunogenic KLH carrier protein, which assists in presenting carbohydrate antigens to the immune system. Indeed, preclinical studies confirmed the modest immunogenicity of our synthetic LewisY–KLH conjugate.

Our long-term fascination with the tumor-associated Globo-H antigen began quite serendipitously. In perusing a 1994 issue of *Helvetica Chimica Acta*, we chanced upon a brief Communication from the laboratory of Giovanni Russo, which described synthetic efforts toward the recently identified, albeit little-known, tumor-associated hexasaccharide, Globo-H.[3] The Globo-H antigen, first isolated from the breast cancer cell line MCF-7 by Hakamori and co-workers,[4] would later be associated with a range of other cancer types. Further work by Colnaghi and associates led to the immunocharacterization of the hexasaccharide via the MBr1 monoclonal antibody.[5] Recognizing the immunological potential of this new class of tumor-associated antigen, we immediately undertook to accomplish a concise and selective chemical synthesis of the Globo-H hexasaccharide. The hope was to conjugate the synthetic glycal to a carrier protein and to evaluate its immunogenicity in preclinical, and ultimately clinical, settings. In 1995, we reported the first chemical synthesis of Globo-H.[6–8] The details of this synthetic effort are presented below.

FIRST-GENERATION MSKCC SYNTHESIS OF GLOBO-H

We first set out to assemble the ABC trisaccharide fragment **1**. As shown in Figure 1, treatment of cyclic carbonate **2** with dimethyldioxirane (DMDO) afforded α-epoxide **3**. The latter was merged with **4**, derived from D-glucal **5**, to provide the AB disaccharide **6**. Benzyl protection of the free hydroxyl, followed by unmasking of the cyclic carbonate, delivered **8**. The latter was selectively converted to **9**, bearing the single C_4' axial hydroxyl that would serve as the site of connection between the B and C rings. The C-ring fluorosugar donor was synthesized in a straightforward fashion. As shown, treatment of **10** with DMDO delivered the α-epoxide **11**, which, upon exposure to tetrabutylammonium fluoride (TBAF), was converted to the requisite β-anomeric fluoride **12**. Benzyl protection of the free hydroxyl group provided **13** in 49% overall yield from **10**. We were now in a position to evaluate the coupling of glycal acceptor **9** and donor **13**. In the event, under Mukayama–Nicolaou conditions, the desired glycosylation proceeded, albeit with modest selectivity, to deliver the trisaccharide as a 3:1 (α/β) mixture of anomers at the B–C ring junction. The desired isomer was isolated in 54% yield. Oxidative removal of the C-ring PMB group afforded the ABC trisaccharide **1**.

DOI: 10.1021/ar5004187
Acc. Chem. Res. 2015, 48, 643–652

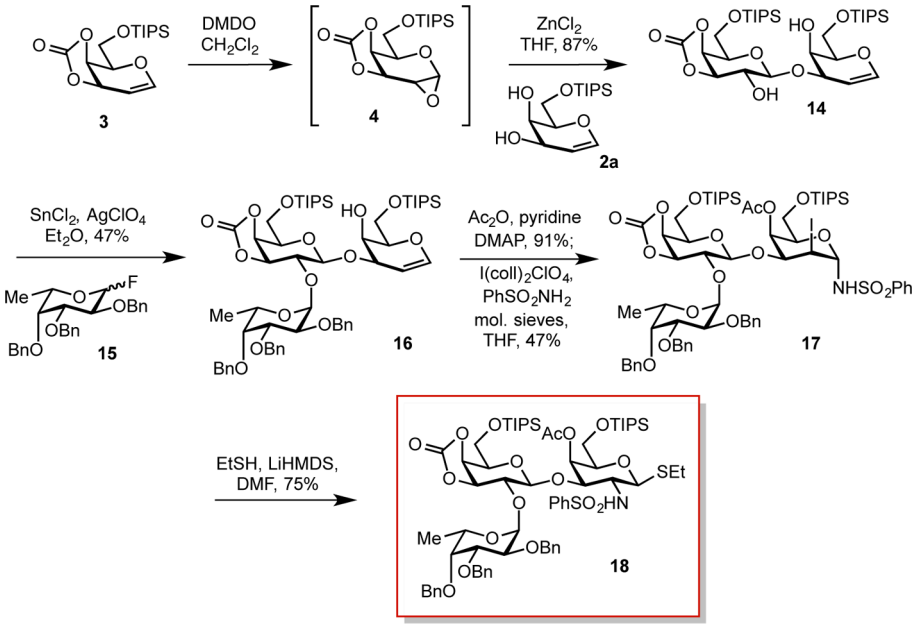


Figure 2. Synthesis of the Globo-H glycal: DEF trisaccharide.

Our route to the DEF trisaccharide coupling partner commenced with the same D-glucal-derived epoxide, **3**. As shown in Figure 2, zinc chloride-mediated coupling of **3** and **2a** proceeded with excellent regioselectivity to afford disaccharide **14**, bearing free hydroxyl groups at C_2' and C_4. At this stage, the hope was to achieve selective fucosylation at the C_2' equatorial position with **15**. We were pleased to find that, upon exposure to Mukayama–Nicolaou conditions, **14** did undergo C_2'-selective fucosylation to deliver trisaccharide **16** as the major product (47% yield). The undesired C_4 fucosylated trisaccharide was isolated as a minor side product (8% yield). Next, acetylation of the free C_4 hydroxyl followed by iodosulfonamidation of the glycal afforded the DEF trisaccharide **17**. It is often possible to achieve direct azaglycosidation of iodo sulfonamide donors; however, in our experience, these "direct rollover" type couplings are typically difficult to achieve with severely hindered substrates, such as the DEF trisaccharide. Accordingly, we elected to pursue a two-step coupling strategy that proceeds via an activated thioglycoside intermediate, **18**. The latter is readily accessible through exposure of **17** to lithium ethanethiolate.

With the ABC and DEF trisaccharide domains in hand, we were now poised to attempt the key coupling en route to the Globo-H hexasaccharide. As shown in Figure 3, upon exposure to methyl triflate, thioglycoside **18** and glycal **1** underwent coupling to deliver a hexasaccharide product in good yield (60%) as a 6:1 ratio of stereoisomers at the C–D ring junction (**19**). However, further structural evaluation revealed that the major product of this reaction was in fact the undesired α-anomer. This result was unanticipated in light of our prior experience with similar systems. Examination of the structural subtleties of our trisaccharide substrates led us to postulate that the undesired product distribution could have arisen from the failure of the sulfonamide to participate in donor activation. If certain inherent structural features of **18** dictated the formation of an active onium species, rather than the desired cyclic sulfonamido species, then it should be possible, through small changes to the DEF donor system, to promote formation of the desired cyclic sulfonamide intermediate en route to the β-anomeric product.

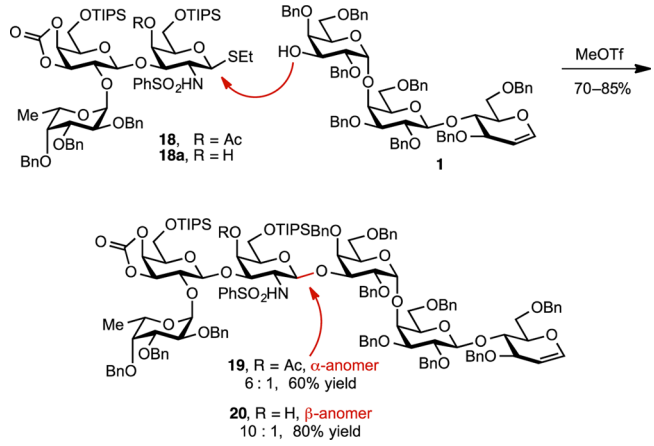


Figure 3. Synthesis of Globo-H glycal: Coupling of ABC and DEF domains.

Along these lines, we sought to probe the impact of modifying the substitution at C_4 on the future D-ring. Specifically, we aimed to replace the C_4 acetate functionality with a free hydroxyl group. We were pleased to observe that methyl triflate-mediated coupling of **18a** (R = H) with **1** proceeded in good yield to generate the hexasaccharide **20** as a 10:1 mixture of isomers, with the β-anomer as the major product. This remarkable result illustrates the impact that small perturbations in the donor ring may have on these types of coupling reactions. By simply replacing an acetate group with a free hydroxyl, we were able to achieve a major reversal in the directionality of this key coupling reaction.

We now launched the final phase of the Globo-H–KLH synthesis effort (Figure 4). Briefly, intermediate **20** was converted to allyl glycoside **21** via a five-step sequence involving (1) global deprotection, (2) peracetylation, (3) epoxidation of the terminal glycal, (4) epoxide opening with allyl alcohol, and (5) removal of the acetate protecting groups. The allyl functional handle was subjected to ozonolysis, and the resultant aldehyde underwent reductive amination with the linker molecule. Finally,

DOI: 10.1021/ar5004187
Acc. Chem. Res. 2015, 48, 643–652

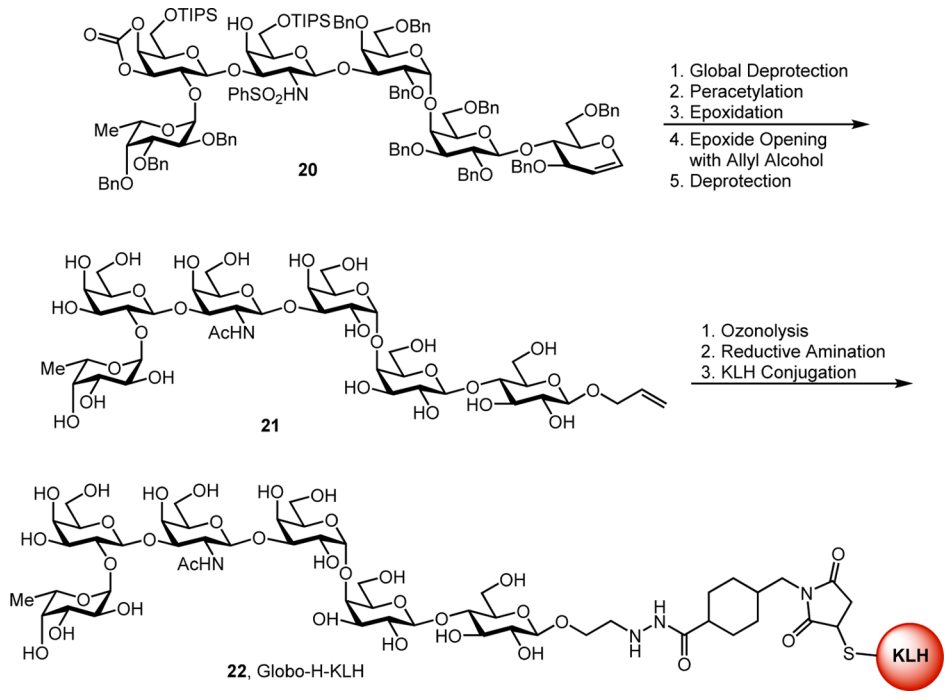


Figure 4. Completion of Globo-H monomer synthesis and conjugation.

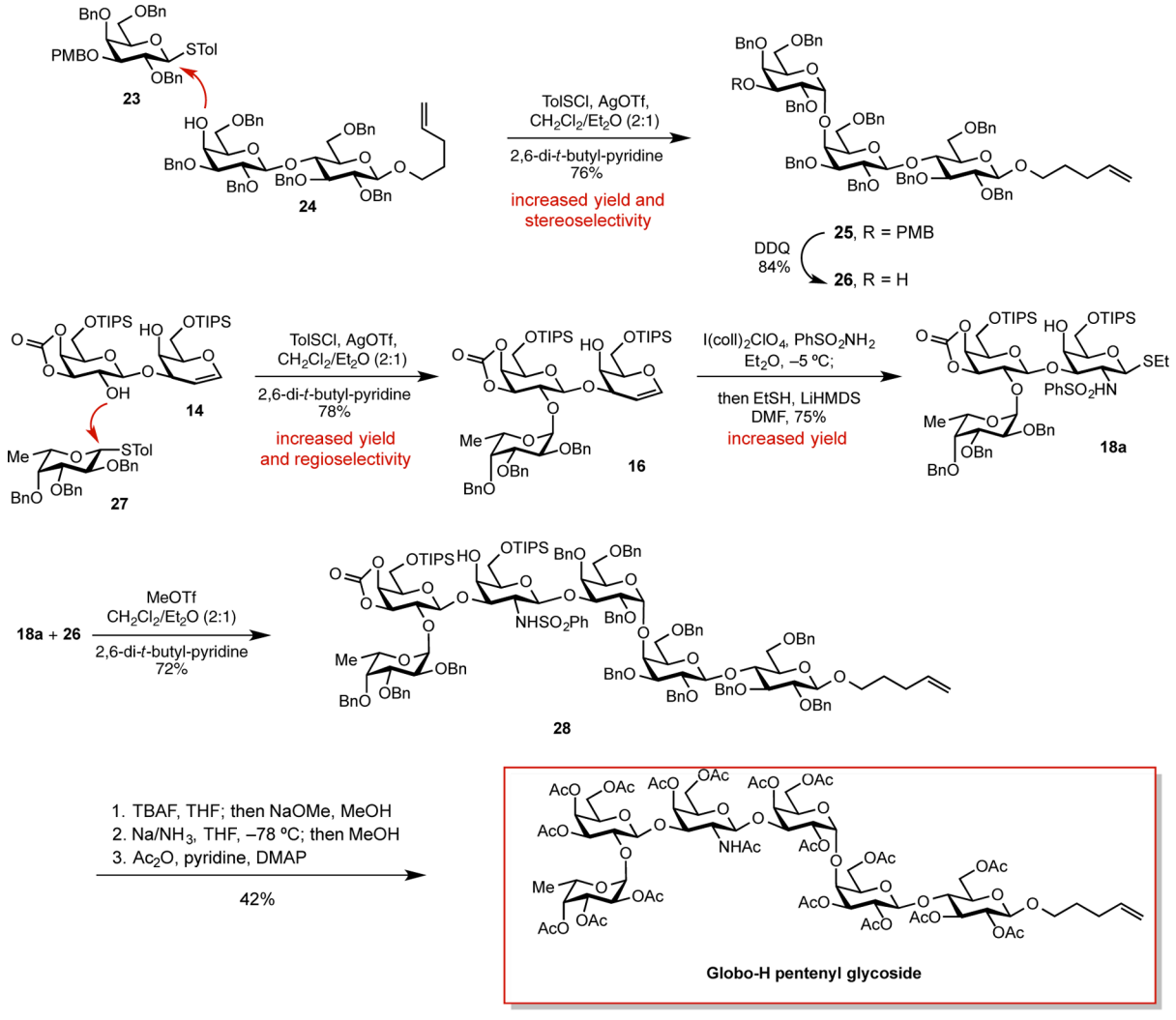


Figure 5. Second-generation synthesis of Globo-H Pentenyl glycoside.

DOI: 10.1021/ar5004187
Acc. Chem. Res. 2015, 48, 643−652

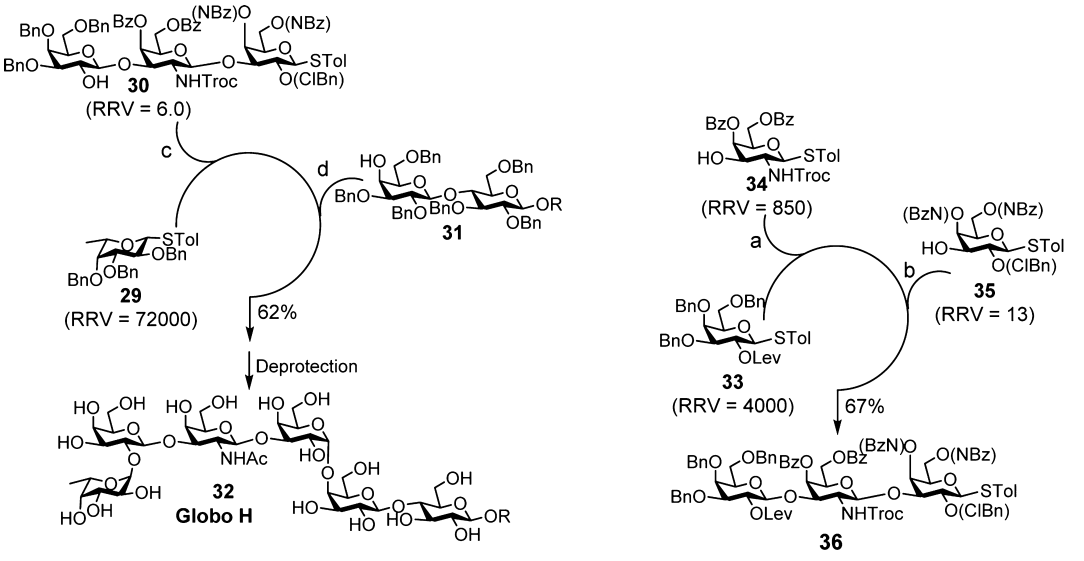


Figure 6. (left) Programmable one-pot synthesis of Globo-H from three building blocks, including the trisaccharide **30**. (right) Programmable one-pot synthesis of the trisaccharide building block **30**.

conjugation to KLH afforded the target Globo-H−KLH vaccine construct **22**.

SECOND-GENERATION MSKCC SYNTHESIS OF GLOBO-H

In 2009, an improved second-generation route was devised that provides rapid access to significant quantities of the Globo-H pentenyl glycoside for incorporation into anticancer vaccine constructs.[9] In this revamped synthesis, we addressed some issues of yield and selectivity that had compromised the overall efficiency of the first-generation route. Specifically, we aimed to improve upon the moderate selectivities observed in the coupling steps en route to the DEF trisaccharide (Figure 2) and the ABC trisaccharide (Figure 1). In the original synthesis, the AB−C galactosylation had proceeded with relatively modest α-stereoselectivities, while the DE−F fucosylation had suffered from incomplete regioselectivity for the C_2' vs C_4 hydroxyl. Drawing inspiration from the Huang synthesis of Globo-H,[10] we explored the use of a preactivated thiogalactosyl donor **23** as a means to achieve stereoselective glycosylation with a prefunctionalized AB acceptor (**24**). Indeed, under modified Huang conditions, we were able to gain access to the desired α-trisaccharide adduct **25** in excellent yield and selectivity. Notably, while the original coupling had required prolonged reaction times (35 h), this transformation was complete within 4 h. Similarly, the coupling of the DE disaccharide **14** with thiofucosyl donor **27** proceeded rapidly to afford the DEF trisaccharide in high yield (78%) with no observed C_4-fucosylated isomer. We also note that under these modified reaction conditions, the yields and selectivities were much more consistent, particularly when the couplings were performed on large (gram) scale.

An optimized procedure was also developed for the synthesis of the DEF thiodonor, **18a**. As shown in Figure 5, minor changes to the solvent system and reaction temperature enabled the ready conversion of **16** to **18a** in 75% overall yield; this represents a significant improvement over the first-generation route, which proceeded in only ∼40% yield over

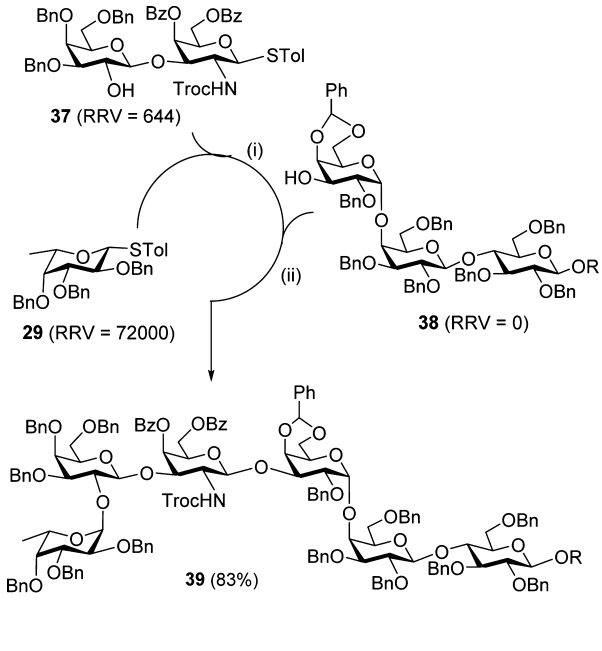


Figure 7. Improved programmable one-pot synthesis of Globo-H to avoid axial glycosidic bond coupling and to increase the overall yield to 83%.

two steps. Finally [3 + 3] coupling and deprotection delivers the Globo-H pentenyl glycoside **28** in good overall yield. Notably, this synthesis directly delivers the hexasaccharide equipped with a pentenyl functional handle for incorporation onto a peptide backbone or conjugation to carrier protein.

GLOBO-H−KLH: PRECLINICAL AND CLINICAL STUDIES

The reported immunological activity of Globo-H−KLH was confirmed in preclinical studies with synthetic material.[11] Upon

DOI: 10.1021/ar5004187
Acc. Chem. Res. 2015, 48, 643−652

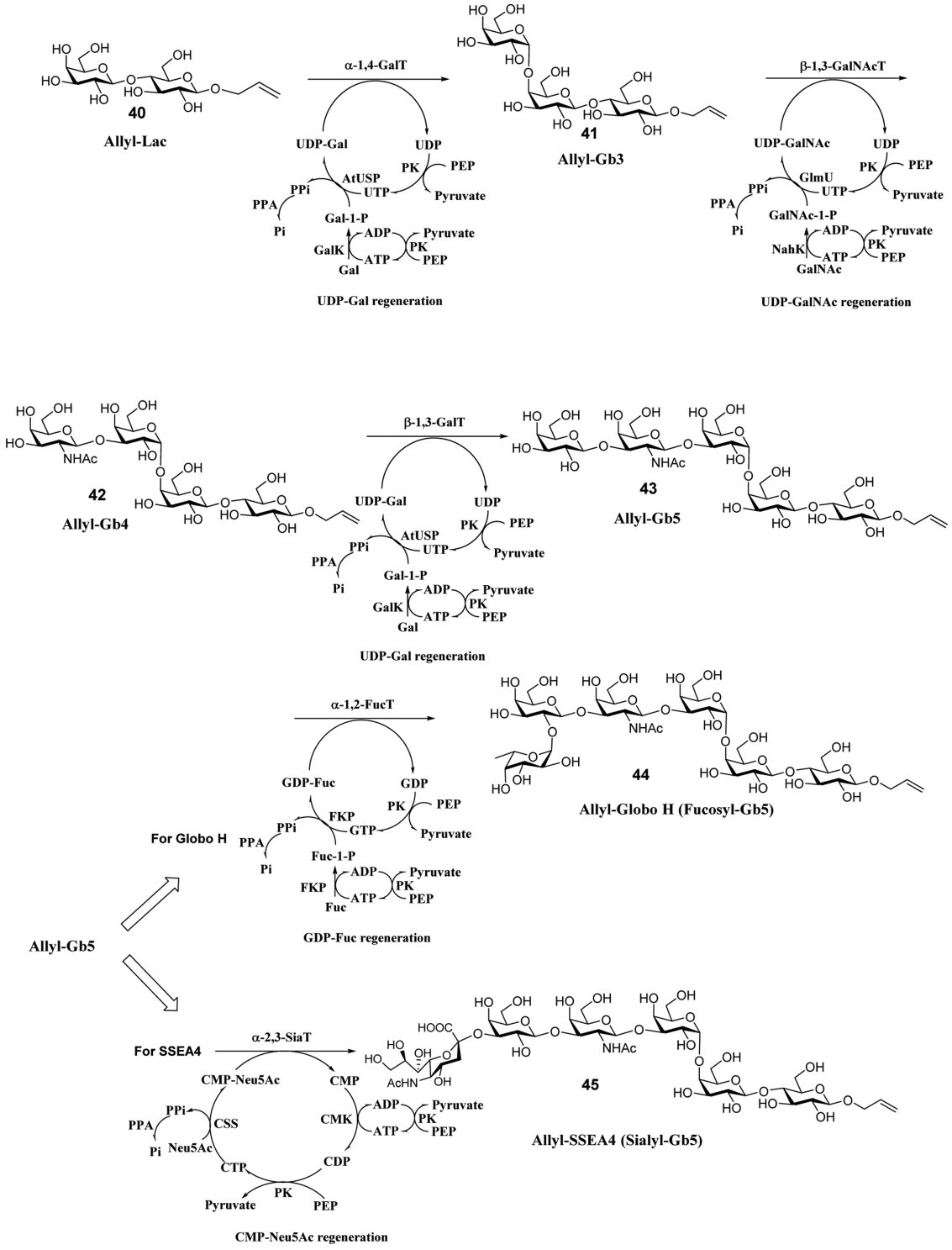


Figure 8. Large-scale enzymatic synthesis of allyl-Globo H and allyl-SSEA-4 using glycosyltransferases coupled with cofactor regeneration. The incorporation of cofactor regeneration into the synthesis is to reduce the cost and eliminate the problem of product inhibition caused by the byproduct of sugar nucleotide cofactor. In every glycosidic bond formation, a monosaccharide is used as starting material for the kinase-catalyzed conversion to the corresponding sugar-1-phosphate followed by enzymatic transformation to the sugar nucleotide cofactor as donor for the glycosyltransferase. Both Globo-H and SSEA4 can be prepared in a two-step reaction in approximately 80% yield.

exposure to synthetic Globo-H−KLH, mice were observed to produce high-titer IgM and modest IgG responses to the Globo-H hexasaccharide. These antibodies were selectively reactive against Globo-H-positive cell lines and were observed to induce complement-mediated lysis of Globo-H-positive MCF-7 cells.

On the basis of these encouraging data, synthetic Globo-H−KLH vaccine candidate was advanced to phase I clinical trials at MSKCC against prostate and breast cancer.[12] These trials established the safety and immunogenicity of the Globo-H−KLH construct; importantly, the magnitude of the immune response was observed to be independent of the level of tumor burden.

DOI: 10.1021/ar5004187
Acc. Chem. Res. 2015, 48, 643−652

The Globo-H–KLH vaccine was then used in combination with QS21, a saponin-based adjuvant, in a phase I clinical trial for metastatic breast cancer.[13] In this study, twenty-seven patients received five vaccinations each over 19 weeks. The vaccine was well-tolerated, and serologic analysis showed the generation of IgM antibodies in most patients, along with a relatively weak IgG response; there was evidence of complement-dependent cytotoxicity in several patients.

PROGRAMMABLE ONE-POT SYNTHESIS OF GLOBO-H

Following the positive results of the phase I trial, this vaccine was outlicensed for further development, and a randomized controlled phase II/III trial was initiated. In order to develop a more cost-effective synthesis of Globo-H under the GMP standard for clinical study, a programmable one-pot synthesis method was adopted (Figure 6),[14] in which some key building blocks were first prepared for the assembly of Globo-H with the allyl group functional handle. The programmable one-pot synthesis method was the first automated method developed to simplify the process and improve the efficiency of oligosaccharide synthesis. With this approach to synthesize a desired oligosaccharide, a database of the relative reactivity value (RRV) of hundreds of thioglycoside building blocks is accessed, and a computer program is used to select and rank the building blocks from the most reactive to the least reactive and to mix them in sequence in the presence of a promoter to obtain the desired product in the best yield. The procedure significantly reduces the time and effort required for the purification and protecting group manipulation steps in each reaction. The first one-pot synthesis of Globo-H shown in Figure 6 involved the use of three building blocks, including a trisaccharide, to produce the protected Globo-H in 62% yield. To obtain the trisaccharide building block, it was synthesized from three monosaccharide building blocks via the one-pot strategy (Figure 6).[14] The synthesis of Globo-H was further improved to 83% yield through development of a second-generation one-pot strategy to eliminate the need for glycosylation at the axial hydroxyl group (Figure 7).[15]

While the phase II/III trial was ongoing at more than 40 clinical centers in Taiwan, India, Korea, Hong Kong, and the United States of America, the development of an enzymatic synthesis of Globo-H was pursued at the same time.

ENZYMATIC SYNTHESIS OF GLOBO-H

In order to further reduce the manufacturing cost and minimize the purification steps and any impurities, a new enzymatic method for the synthesis of Globo-H was developed after identification of the enzymes to be used in each glycosidic bond formation. This enzymatic method was based on the use of glycosyltransferases coupled with cofactor regeneration (Figure 8),[16] a process developed to reduce the cost of sugar nucleotide cofactor and eliminate the problem of product inhibition caused by the byproduct of sugar nucleotide (Figure 8). This strategy was proven to be suitable for the synthesis of oligosaccharides on large scales (see ref 16 for citations). All the enzymes used in the synthesis were first identified from different microorganisms and then overexpressed in *Escherichia coli*. Most notably, the cofactor regeneration steps involve the use of a hexose kinase to generate the corresponding sugar-1-phosphate for further conversion to the sugar nucleotide as donor for the glycosyltransferase. This enzymatic process of Globo-H synthesis from allyl lactose required only two steps with over 80% yield and reduced the cost of production substantially, thus enabling the manufacture of Globo-H on large scales for the multicenter late-stage human trials.

DISTRIBUTION OF GLOBO-SERIES GLYCANS (GLOBO-H, SSEA3, AND SSEA4) ON BREAST CANCER CELLS, CANCER STEM CELLS, AND OTHER TYPES OF CANCER CELLS

Since Globo-H is only expressed on cancer cells and not detectable on normal cells, the Globo-H vaccine, in principle, should be effective against any type of cancer, as long as the cancer cell surface is Globo-H positive. Indeed, at least 16 different types of epithelial cancers have been found to be Globo-H positive, including those in 60–65% of breast cancer patients.[17] With the monoclonal antibody VK9 and glycan arrays available for detection of the presence of Globo-H on tissues and anti-Globo-H antibody in sera, we have developed a sensitive diagnostic tool for use to identify the patients with Globo-H expression and provide an option for therapeutic intervention.[17] In addition to Globo-H, we have also found that both breast cancer cells and cancer stem cells also express SSEA3 (Gb5) and SSEA4 on the surface,[18] while these three glycans are not detectable on normal cells (Figure 9). Further studies showed that these three glycan markers were also present on the cell surface of 15 other types of cancers,[19] suggesting a possible broad application of the vaccine.

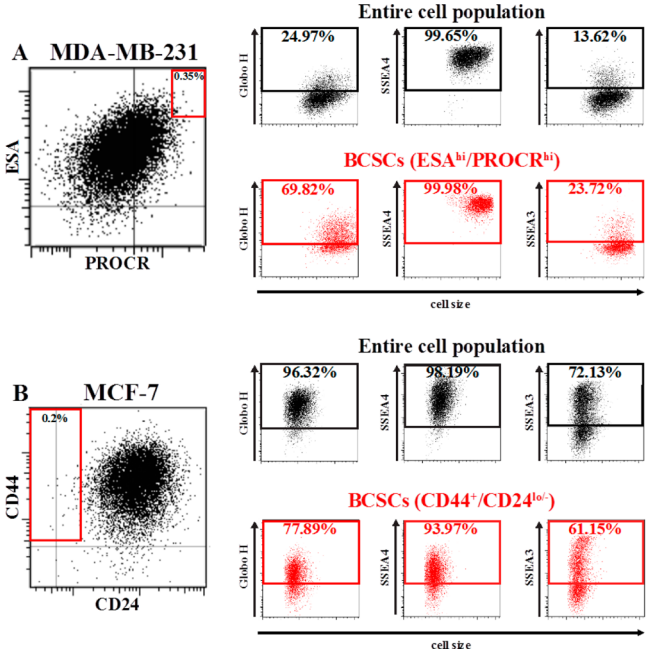


Figure 9. Distribution of GloboH, SSEA3 (Gb5), and SSEA4 on the surface of entire breast cancer cell populations and breast cancer stem cells (BCSC). In these analyses, the antibodies against each of the three globo-series glycans (Globo-H, SSEA4, and SSEA3) were used for detection of the glycan markers. Two different sets of breast cancer stem cell markers and their antibodies were used for cancer stem cell sorting. The result showed that all the three glycans were on the surface of cancer cells and cancer stem cells, and the level of SSEA4 expression was the most significant (>94%).

DOI: 10.1021/ar5004187
Acc. Chem. Res. 2015, 48, 643–652

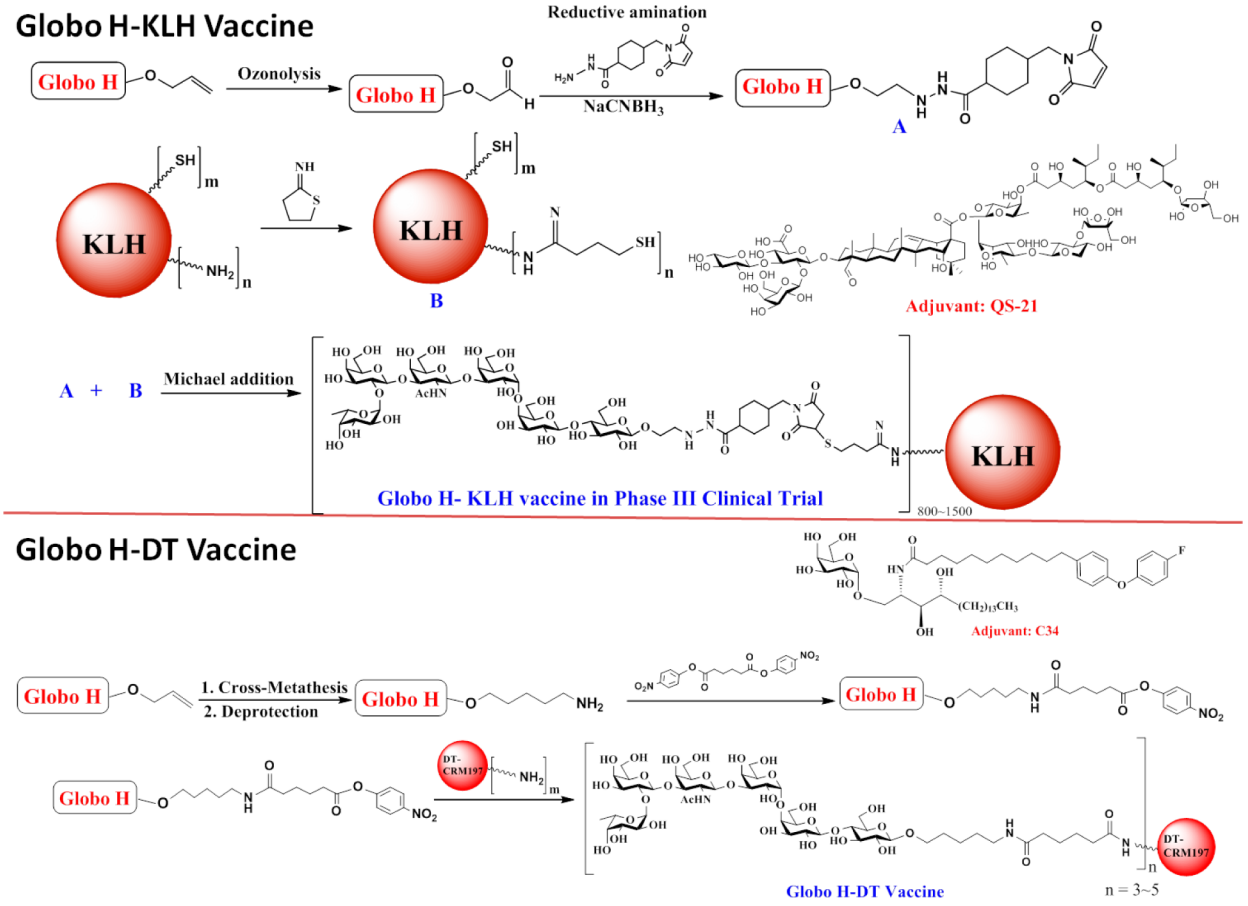


Figure 10. (top) Preparation of Globo-H vaccine using KLH as carrier and QS21 as adjuvant. (bottom) Preparation of a new generation Globo-H vaccine using the carrier CRM 197 in combination with the adjuvant C34, a ligand for CD1d on dendritic cells and B cells, to activate T cells and induce a class switch.

A NEW GENERATION GLOBO-H VACCINE

Based on the limited number of samples used in the analysis, it was found that more than 98% of the breast cancer patients have at least one of the three glycans expressed on the surface of cancer cells and the cancer stem cells.[18] This finding is significant, because if a vaccine is available to target these three unique glycans, most breast cancer patients could be treated without screen. In addition, carbohydrate-based vaccines often generate mainly IgM antibodies, due to the short-term T-cell independent immune response through specific B cells. Achieving the more robust and long-term T-cell dependent response and IgG production is a major challenge and requires T-cell participation through cytokine regulation. With these goals in mind, we have launched the development of a second generation breast cancer vaccine, which was expected to have a long-term memory and elicit significant IgG antibody response against the three unique glycans. We thus decided to test different carriers and adjuvants and found that when Globo-H was conjugated to the diphtheria toxin mutant CRM197 and used together with the glycolipid adjuvant C34 (Figure 10), a designed ligand for the CD1d receptor on dendritic cells and B cells, the vaccine gave the best immune response. It was believed that when the adjuvant was presented by CD1d to the NKT-cell receptor, the NKT cell was activated more selectively toward the Th1 than the Th2 response, as measured by the level of secreted IFN gamma and IL4 cytokines; this adjuvant effect resulted in a significant class switch with more IgG antibody response against these three glycan epitopes as shown by the glycan array analysis of the serum from immunized mice.[18] The immunology profile is thus much improved over that of the first generation vaccine based on Globo-H–KLH–QS21 (Figure 11). This new vaccine was also outlicensed for clinical evaluation of breast cancer and other types of cancers, including, for example, pancreatic, ovarian, colon, lung, gastric, and liver cancers, and for consideration of early stage treatment and even preventive application.

ROLES OF GLOBO-H, SSEA4, AND SSEA3 AND FUTURE PERSPECTIVE

The functional mechanism of the globo-series glycosphingolipids (i.e., Globo-H, SSEA4, and SSEA3) associated with tumor metastasis is still not well understood. However, these three glycolipids with SSEA3 as common epitope were first found on embryonic stem cells, then disappeared until cancer cells appear. Though the functions of the globo-series glycolipids are not clear, their unique presence on cancer cells and cancer stem cells makes them better targets for vaccine design than the other carbohydrate-based antigens, most of which are just overexpressed on cancer cells. To search for the binding proteins of globo-series glycolipids, recent studies using glycans conjugated to magnetic nanoparticles have identified FKBP4 as the binding protein of SSEA4, and this protein was found to be associated with the transport of SSEA4 to the cell surface.[20] The major binding protein for Globo-H was also identified (Artmin) but its role in cancer progression is unclear. In

DOI: 10.1021/ar5004187
Acc. Chem. Res. 2015, 48, 643−652

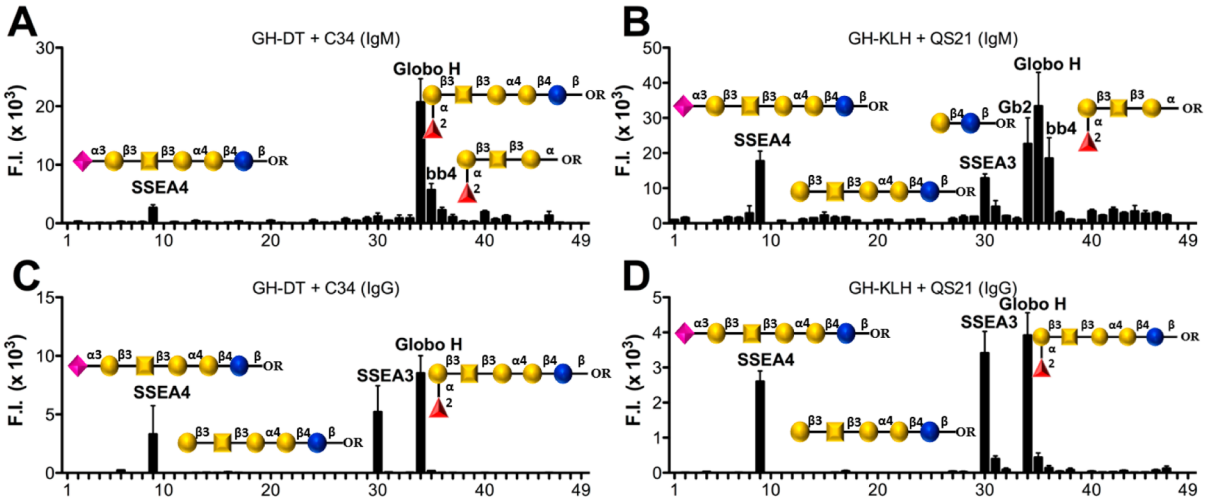


Figure 11. Glycan array analysis of the serum from mice immunized with Globo-H−KLH−QS21 and Globo-H−DT−C34. As shown, the Globo-H−DT−C34 vaccine induces a higher level of IgG antibodies and is more selective for the globo-series glycans (i.e., Globo-H, SSEA3, and SSEA3).

addition, the mechanism of action of the Globo-H vaccine, including its uptake, processing, and presentation in the immune system, remains to be investigated in order to elucidate the process of immune response and cross reactivity toward these three unique glycan epitopes. Answering all of these issues would provide a better understanding of the globo-series glycans in cancer progression and their application to cancer vaccine design. In addition to the vaccine approach, the antibodies against the three globo-series glycans have been shown to be effective as anticancer agents.[17−19] It is therefore also worth of the effort to pursue an alternative anticancer strategy to target the enzymes involved in the biosynthesis of the globo-series glycolipids, especially the enzyme galactosyltransferase V responsible for the synthesis of SSEA3, and to investigate the possible effect of PD1 and CTLA4 on these anticancer agents.

AUTHOR INFORMATION

Corresponding Authors

*S.J.D. E-mail: s-danishefsky@ski.mskcc.org.
*C.H.W. E-mail: chwong@gate.sinica.edu.tw.

Notes

The authors declare no competing financial interest.

Biographies

Samuel J. Danishefsky completed his B.S. at Yeshiva University and his Ph.D. at Harvard University with Peter Yates. Following postdoctoral studies at Columbia University with Gilbert Stork, he began his independent academic career at the University of Pittsburgh, where he became Professor in 1971. In 1980, he moved to Yale University but returned to New York in 1993 as Professor of Chemistry at Columbia University and Kettering Professor at Memorial Sloan Kettering Cancer Center. His research interests include synthesis of natural products and biologic agents. Professor Danishefsky has received numerous honors, including the Wolf Prize in Chemistry, the ACS Cope Medal, the H. C. Brown Award, the Benjamin Franklin Award, the NAS Award in the Chemical Sciences, and the Ralph Hirschmann Award in Peptide Chemistry. He is a member of the National Academy of Sciences.

Youe-Kong Shue, Vice Chairman of OBI Pharma, Inc., currently also serves as the Principal of Global Clinical and Regulatory Planning for the company. He was the President and CEO of OBI from November 2009 to April 2013. At Optimer, he led the development of fidaxomicin, the first new antibiotic to be approved in over 30 years for the treatment of *Clostridium difficile*-associated diarrhea (CDAD), received US FDA and EMEA approval in 2011. Dr. Shue began his career in 1983 at Abbott Laboratories, where he conducted neuroscience research and later at Cubist and AstraZeneca. He received his B.A. from National Cheng Kung University, M.A. from National Taiwan University, and Ph.D. in Organic Chemistry from the University of Pittsburgh. He conducted a postdoctoral research at MIT before joining Abbott Laboratories. Dr. Shue holds more than 50 patents and has published more than 30 peer reviewed publications.

Michael N. Chang received his B.S. in Chemistry from Fu Jen Catholic University and Ph.D. in Organic Chemistry from Brandeis University and conducted postdoctoral research at MIT. He started his career at Merck, Sharpe & Dohme and later at Rhone-Poulenc Rorer. Before founding Pharmanex, he worked for 15 years in the pharmaceutical industry. In 1993, he established Cinogen, a manufacturing company, and Pharmanex, Inc., a health supplement products company, later acquired by NuSkin Enterprises. He founded Optimer Pharmaceuticals, Inc., in 1999, and served as its president and CEO, which was acquired by Cubist Pharmaceuticals in 2012. He is currently serving as Chairman of OBI Pharma, Inc., and a director of Amaran Biotech Inc.

Chi-Huey Wong received his B.S. and M.S. degrees from National Taiwan University and Ph.D. in Chemistry from MIT. After his postdoctoral research with Whitesides, he became a faculty member of Texas A&M University and then in 1989 Professor and Ernest W. Hahn Chair in Chemistry at the Scripps Research Institute. He is currently also serving as President of Academia Sinica in Taipei. His research interests include development of chemical and enzymatic methods in organic synthesis, carbohydrate chemistry, and biology and drug design. He received many honors, including the Roy Whistler Award of International Carbohydrate Organization, the ACS Claude S. Hudson Award in Carbohydrate Chemistry, the International Enzyme Engineering Award, the ACS Award for Creative Work in Synthetic Organic Chemistry, and the AC Cope Medal, the Presidential Green Chemistry Award, the Nikkei Asia Prize, and the Wolf Prize in Chemistry. He is a member of the National Academy of Sciences.

DOI: 10.1021/ar5004187
Acc. Chem. Res. 2015, 48, 643−652

REFERENCES

(1) (a) Zhang, S.; Cordon-Cardo, C.; Zhang, H. S.; Reuter, V. E.; Adluri, S.; Hamilton, W. B.; Lloyd, K. O.; Livingston, P. O. Selection of tumor antigens as targets for immune attack using immunohistochemistry: I. Focus on gangliosides. *Int. J. Cancer* **1997**, *73*, 42−49. (b) Zhang, S.; Zhang, H. S.; Cordon-Cardo, C.; Reuter, V. E.; Singhal, A. K.; Lloyd, K. O.; Livingston, P. O. Selection of tumor antigens as targets for immune attack using immunohistochemistry: II. Blood group-related antigens. *Int. J. Cancer* **1997**, *73*, 50−56. (c) Zhang, S.; Zhang, H. S.; Reuter, V. E.; Slovin, S. F.; Scher, H. I.; Livingston, P. O. Expression of potential target antigens for immunotherapy on primary and metastatic prostate cancers. *Clin. Cancer Res.* **1998**, *4*, 295−306.

(2) (a) Livingston, P. O.; Wong, G. Y. C.; Adluri, S.; Tao, Y.; Padavan, M.; Parente, R.; Hanlon, C.; Calves, M. J.; Helling, F.; Ritter, G.; Oettgen, H. F.; Old, L. J. Improved survival in stage III melanoma patients with GM2 antibodies: a randomized trial of adjuvant vaccination with GM2 ganglioside. *J. Clin. Oncol.* **1994**, *12*, 1036−44. (b) Taniguchi, N., Endo, T., Hart, G. W., Seeberger, P. H., Wong, C.-H., Eds. *Glycoscience: Biology and Medicine*; Springer: Tokyo, 2015; pp 1−1556.

(3) Lay, L.; Nicotra, F.; Panza, L.; Russo, G.; Adobati, E. Oligosaccharides related to tumor-associated antigens. Part I. Synthesis of the propyl glycoside of the trisaccharide α-L-Fucp-(1 → 2)-β-D-Galp-(1 → 3)-β-D-GalpNAc, component of a tumor antigen recognized by the antibody MBr1. *Helv. Chim. Acta* **1994**, *77*, 509−514.

(4) (a) Kannagi, R.; Levery, S. B.; Ishijamik, F.; Hakomori, S.; Schevinsky, L. H.; Knowles, B. B.; Solter, D. New globoseries glycosphingolipids in human teratocarcinoma reactive with the monoclonal antibody directed to a developmentally regulated antigen, stage-specific embryonic antigen 3. *J. Biol. Chem.* **1983**, *258*, 8934−8942. (b) Bremer, E. G.; Levery, S. B.; Sonnino, S.; Ghidoni, R.; Canevari, S.; Kannagi, R.; Hakomori, S. Characterization of a glycosphingolipid antigen defined by the monoclonal antibody MBr1 expressed in normal and neoplastic epithelial cells of human mammary gland. *J. Biol. Chem.* **1984**, *259*, 14773−14777.

(5) Menard, S.; Tagliabue, E.; Canevari, S.; Fossati, G.; Colnaghi, M. I. Generation of monoclonal antibodies reacting with normal and cancer cells of human breast. *Cancer Res.* **1983**, *43*, 1295−1300.

(6) Bilodeau, M. T.; Park, T. K.; Hu, S.; Randolph, J. T.; Danishefsky, S. J.; Livingston, P. O.; Zhang, S. Total Synthesis of a human breast tumor associated antigen. *J. Am. Chem. Soc.* **1995**, *117*, 7840−7841.

(7) Park, T. K.; Kim, I. J.; Hu, S. H.; Bilodeau, M. T.; Randolph, J. T.; Kwon, O.; Danishefsky, S. J. Total synthesis and proof of structure of a human breast tumor (Globo-H) antigen. *J. Am. Chem. Soc.* **1996**, *118*, 11488−11500.

(8) Allen, J. R.; Allen, J. G.; Zhang, X. F.; Williams, L. J.; Zatorski, I.; Ragupathi, G.; Livingston, P. O.; Danishefsky, S. J. A second generation synthesis of the MBr1 (Globo-H) breast tumor antigen: New application of the n-pentenyl glycoside method for achieving complex carbohydrate protein linkages. *Chem.—Eur. J.* **2000**, *6*, 1366−1375.

(9) Jeon, I.; Iyer, K.; Danishefsky, S. J. A practical total synthesis of Globo-H for use in anticancer vaccines. *J. Org. Chem.* **2009**, *74*, 8452−8455.

(10) Wang, Z.; Zhou, L.; El-Boubbou, K.; Ye, X.-S.; Huang, X. Multicomponent one-pot synthesis of the tumor-associated carbohydrate antigen Globo-H based on preactivation of thioglycosyl donors. *J. Org. Chem.* **2007**, *72*, 6409−6420.

(11) Ragupathi, G.; Park, T. K.; Zhang, S. L.; Kim, I. J.; Graber, L.; Adluri, S.; Lloyd, K. O.; Danishefsky, S. J.; Livingston, P. O. A practical total synthesis of Globo-H for use in anticancer vaccines. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 125−128.

(12) Slovin, S. F.; Ragupathi, G.; Adluri, S.; Ungers, G.; Terry, K.; Kim, S.; Spassova, M.; Bornmann, W. G.; Fazzari, M.; Dantis, L.; Olkiewicz, K.; Lloyd, K. O.; Livingston, P. O. Carbohydrate vaccines in cancer: Immunogenicity of a fully synthetic globo H hexasaccharide conjugate in man. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 5710−5715.

(13) Gilewski, T.; Ragupathi, G.; Bhuta, S.; Williams, L. J.; Musselli, C.; Zhang, X.-F.; Bencsath, K. P.; Panageas, K. S.; Chin, J.; Norton, L.; Houghton, A. N.; Livingston, P. O.; Danishefsky, S. J. Immunization of metastatic breast cancer patients with a fully synthetic globo H conjugate: A phase I trial. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 3270−3275.

(14) Burkhart, F.; Zhang, Z.; Wacowich-Sagarbi, S.; Wong, C.-H. Synthesis of the Globo H hexasaccharide using the programmable reactivity-based one-pot strategy. *Angew. Chem., Int. Ed.* **2001**, *40*, 1274−76.

(15) Chang, S. H.; Han, J.-L.; Tseng, S. Y.; Lee, H.-Y.; Lin, C.-W.; Lin, Y.-C.; Jeng, W.-Y.; Wang, A. H. J.; Wu, C.-Y.; Wong, C.-H. Glycan array on aluminum oxide-coated glass slides through phosphonate chemistry. *J. Am. Chem. Soc.* **2010**, *132*, 13371−13380.

(16) Tsai, T.-I.; Lee, H.-Y.; Chang, S.-H.; Wang, C.-H.; Tu, Y.-C.; Lin, Y.-C.; Hwang, D.-R.; Wu, C.-Y.; Wong, C.-H. Effective sugar nucleotide regeneration for the large-scale enzymatic synthesis of Globo H and SSEA4. *J. Am. Chem. Soc.* **2013**, *135*, 14831−14839.

(17) Wang, C.-C.; Huang, Y.-L.; Ren, C.-T.; Lin, C.-W.; Hung, J.-T.; Yu, J.-C.; Yu, A.; Wu, C.-Y.; Wong, C.-H. Glycan microarray of Globo H and related structures for quantitative analysis of breast cancer. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 11661−11666.

(18) Huang, Y.-L.; Hung, J.-T.; Cheung, S. K. C.; Lee, H.-Y.; Chu, K.-C.; Li, S.-T.; Lin, Y.-C.; Ren, C.-T.; Cheng, T.-J.; Hsu, T.-L.; Yu, A. L.; Wu, C.-Y.; Wong, C.-H. Carbohydrate-based vaccines with a glycolipid adjuvant for breast cancer. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 2517−2522.

(19) Lou, Y.-W.; Wang, P.-Y.; Yeh, S.-C.; Chuang, P.-K.; Li, S.-T.; Wu, C.-Y.; Khoo, K.-H.; Hsiao, M.; Hsu, T.-L.; Wong, C.-H. Stage-specific embryonic antigen-4 as a potential therapeutic target in glioblastoma multiforme and other cancers. *Proc. Natl. Acad. Sci. U.S.A.* **2014**, *111*, 2482−2487.

(20) Hung, T.-C.; Lin, C.-W.; Hsu, T.-L.; Wu, C.-Y.; Wong, C.-H. Investigation of SSEA-4 Binding Protein in Breast Cancer Cells. *J. Am. Chem. Soc.* **2013**, *135*, 5934−5937.

DOI: 10.1021/ar5004187
Acc. Chem. Res. 2015, 48, 643−652