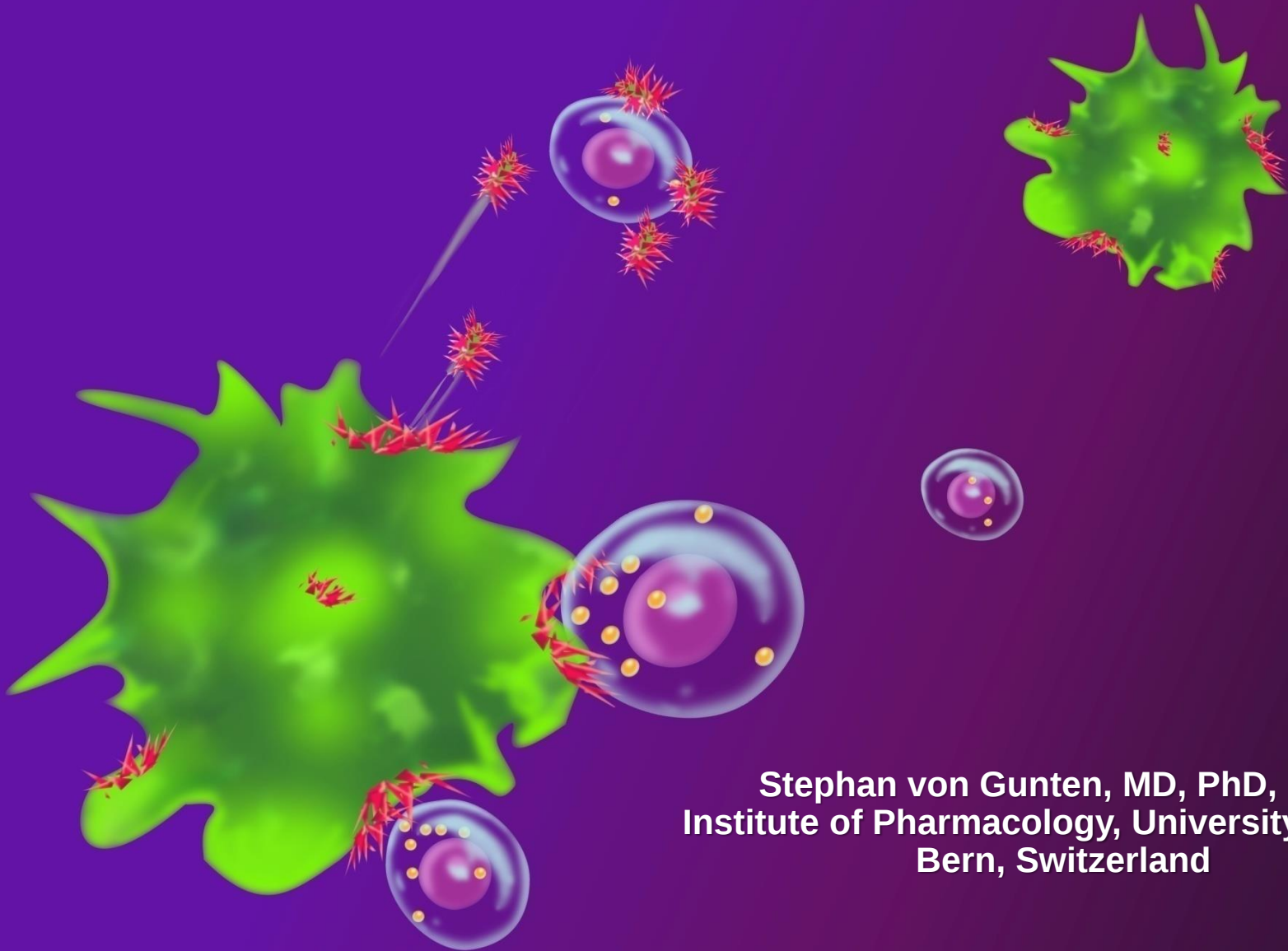
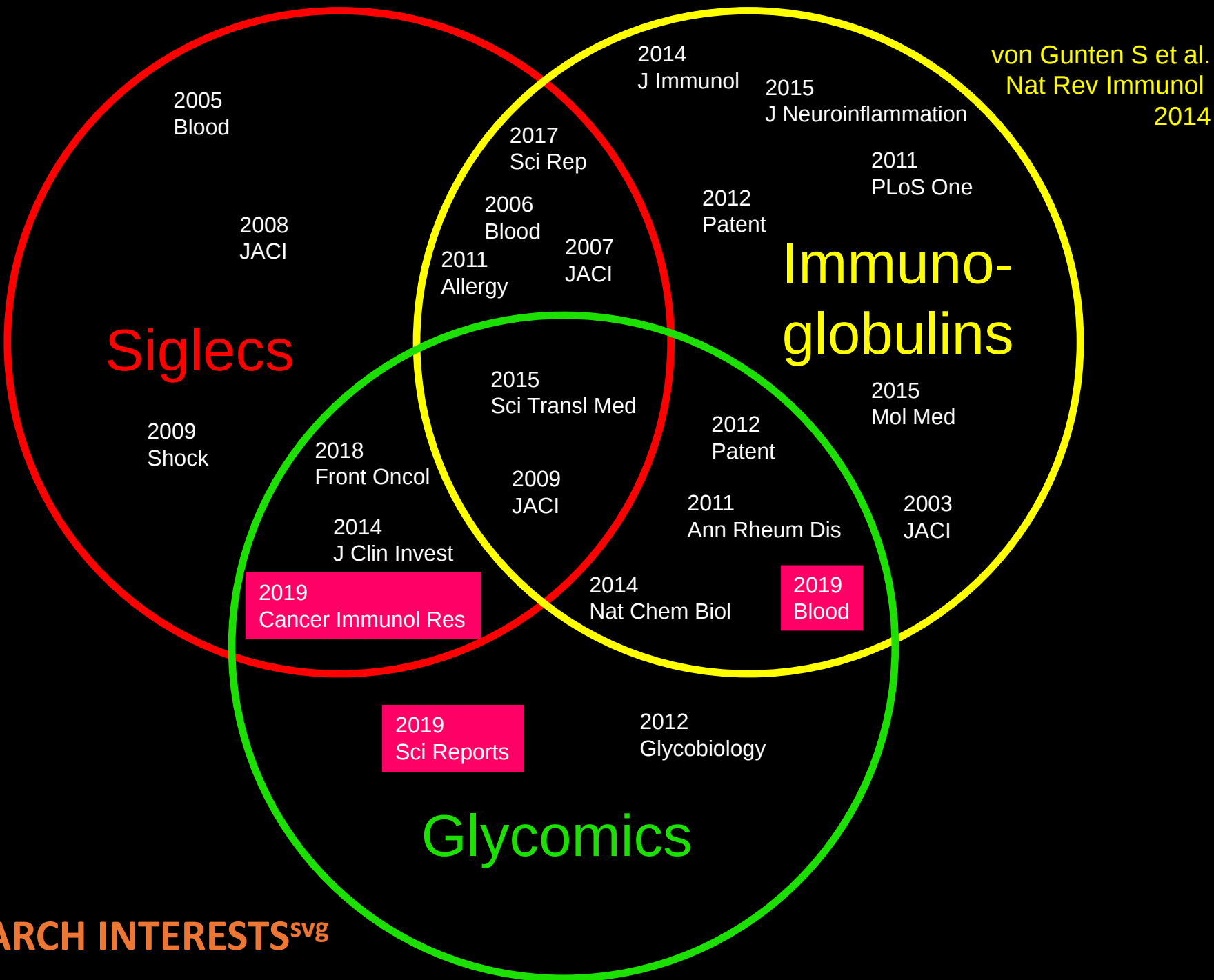


Glycoimmunology – vaccines, tumor glycosylation

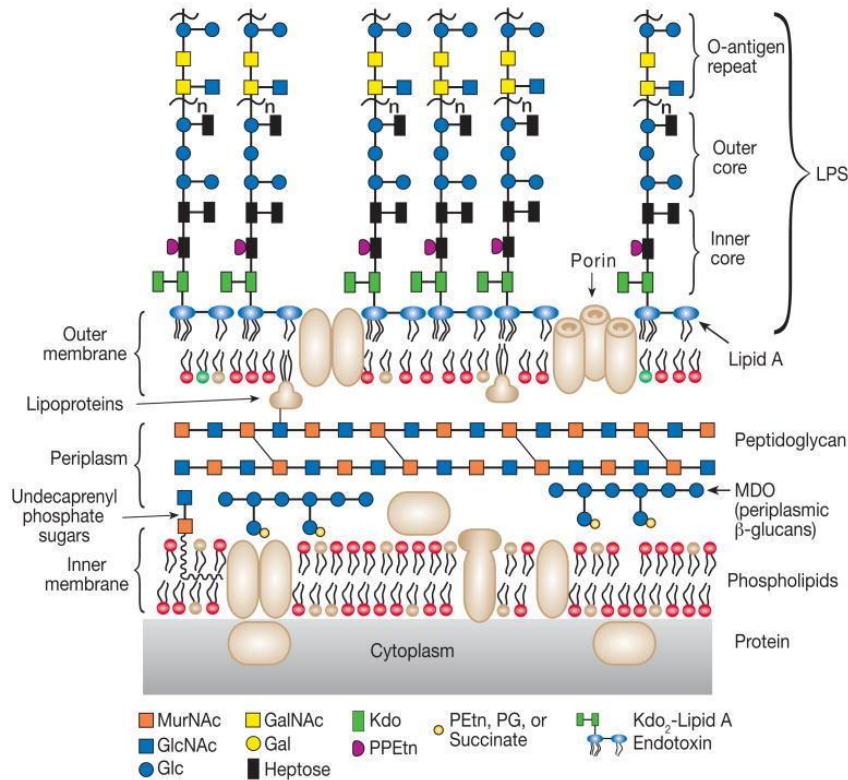


Stephan von Gunten, MD, PhD, MME
Institute of Pharmacology, University of Bern,
Bern, Switzerland

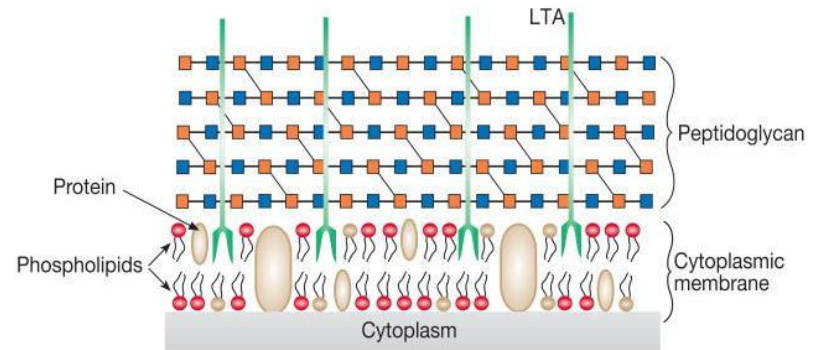


Bacterial glycans

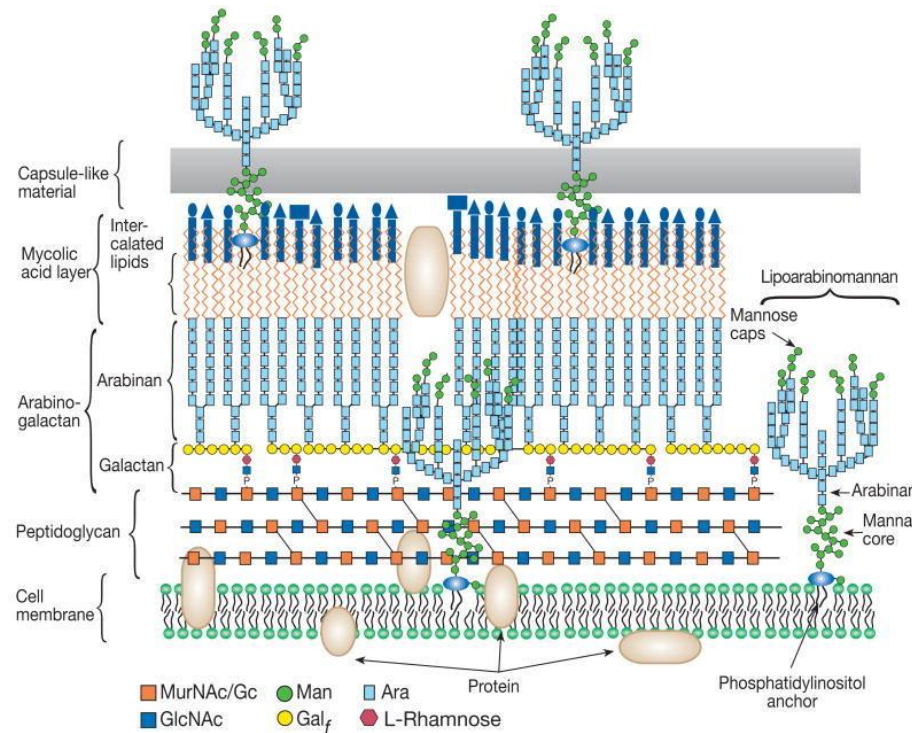
Gram-negative bacteria



Gram-positive bacteria



Mycobacteria



Esko JD, Doering TL, Raetz CRH.

In: *Essentials of Glycobiology*. Cold Spring Harbor (NY); 2009

Galectins (Gal β -binding lectins; formerly S-type lectins)

Geodia cydonium



Zebrafish

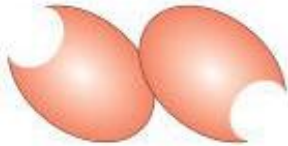


Xenopus laevis



Humans

Prototypical



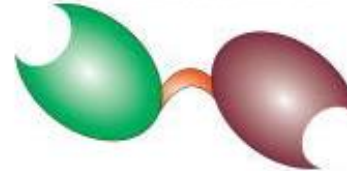
Galectin-1
Galectin-2
Galectin-7
Galectin-10
Galectin-13
Galectin-14

Chimeric



Galectin-3

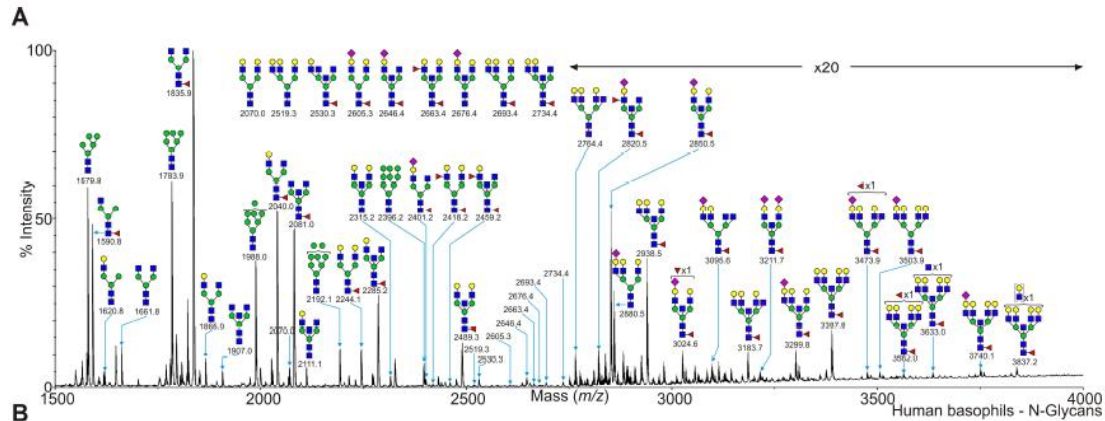
Tandem repeat



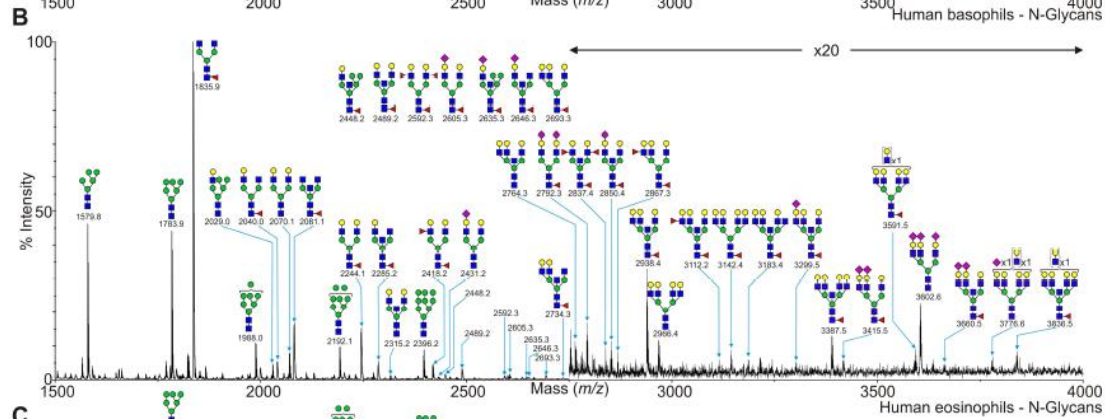
Galectin-4
Galectin-8
Galectin-9
Galectin-12

Glycocalyx: diversity

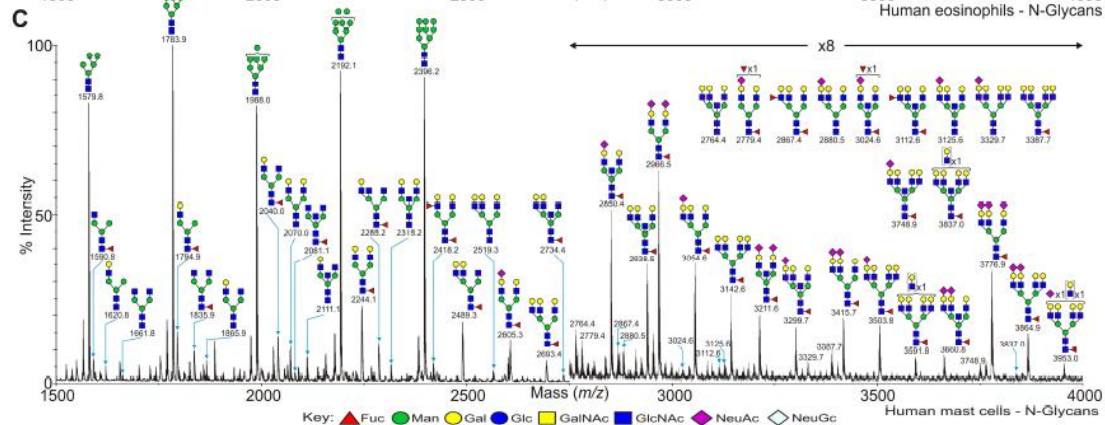
Basophils



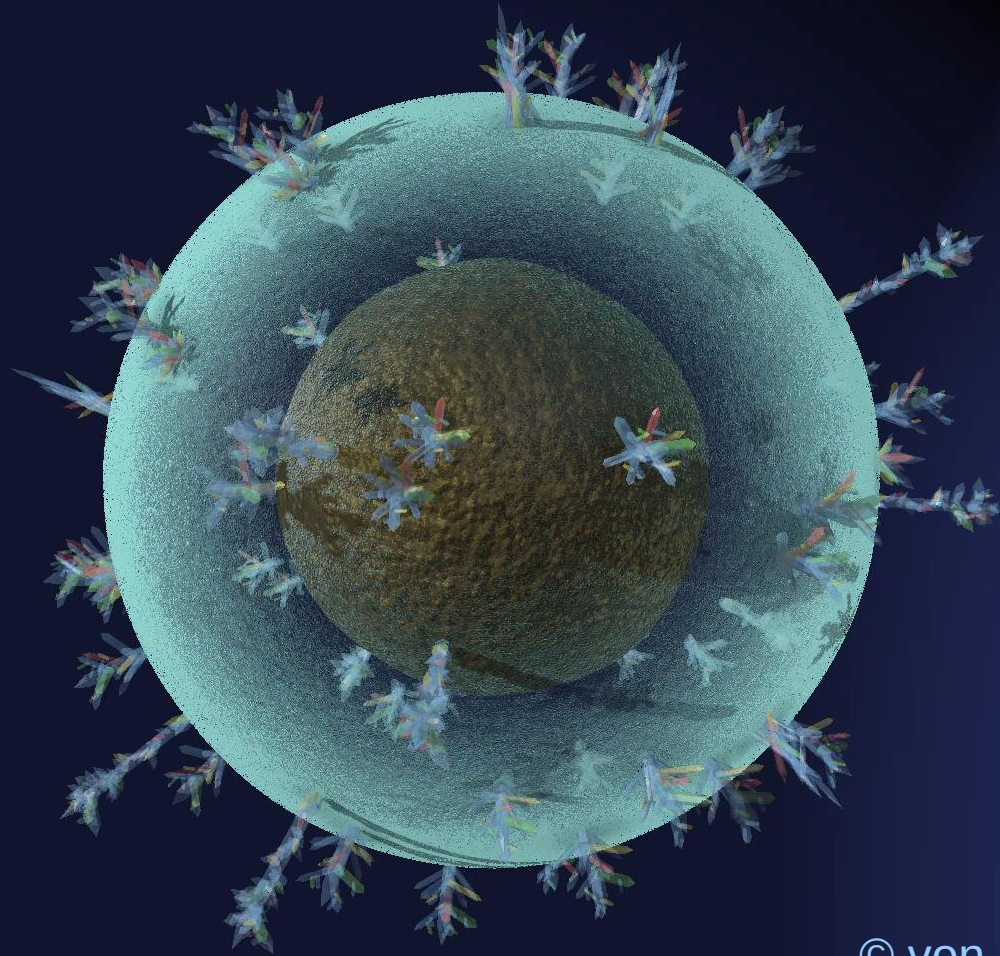
Eosinophils



Mast cells



North SJ, von
Gunten S et al.
Glycobiology 2011



© von Gunten Laboratory

Carbohydrate-based vaccines in development

Infectious disease

Indication	Vaccine	Phase of development
Enterohaemorrhagic <i>Escherichia coli</i>	O-specific polysaccharide–protein conjugate	Phase I ¹⁸²
Group A <i>Streptococcus</i> spp.	Glycoconjugate of Group A polysaccharide with TT	Preclinical ¹⁸³
Group B <i>Streptococcus</i> spp.	Glycoconjugates of type Ia, Ib, II, III and V polysaccharides linked to carrier proteins	Phase II ¹⁸⁴
<i>Haemophilus influenzae</i> (non-typeable)	Subunit–detoxified lipooligosaccharide conjugate	Preclinical ¹⁸⁵
<i>Pseudomonas aeruginosa</i>	Octavalent glycoconjugate of O-polysaccharide with toxin A	Phase III ¹⁸⁶
<i>Salmonella typhi</i>	rEPA–Vi conjugate vaccine	Phase III ^{133,187}
<i>Shigella dysenteriae</i>	O-specific polysaccharide–protein conjugate	Preclinical ⁴²
<i>Shigella flexneri</i>	O-specific polysaccharide–protein conjugate	Phase II ¹⁸⁸
<i>Shigella sonnei</i>	O-specific polysaccharide–protein conjugate	Phase III ¹⁸⁹
<i>Streptococcus pneumoniae</i>	Glycoconjugates of synthetic 6B polysaccharide motifs	Preclinical ⁴³
<i>Vibrio cholerae</i>	Lipopolysaccharide–protein conjugate	Phase I ¹⁹⁰
<i>Aspergillus fumigatus</i>	β-Glucan–CRM197 conjugate	Preclinical ^{65,66}
<i>Candida albicans</i>	Cell surface oligomannosyl epitope (various conjugates)	Preclinical ^{63,64}
	β-glucan–CRM197 conjugate	Preclinical ^{65,66}
<i>Cryptococcus neoformans</i>	Glycoconjugate of capsular polysaccharide with TT	Phase I ⁵⁰
	β-glucan–CRM197 conjugate	Preclinical ^{65,66}
<i>Leishmania</i> spp.	Lipophosphoglycan	Preclinical ⁹⁷
	Lipophosphoglycan conjugates	Preclinical ¹⁰³
<i>Plasmodium falciparum</i>	Glycosylphosphatidylinositol–KLH conjugate	Preclinical ⁹³
HIV-1	Man α(1→2)Man oligomannosyl epitope (various conjugates, engineered yeast strains and modified glycoproteins)	Preclinical ^{17,113–118,165}
Breast cancer	Unimolecular hexavalent conjugates (Globo H–GM2–Lewis ^x –sTn–TF–Tn–R)	Preclinical ¹⁴⁹
	sTn(c)–KLH plus QS-21 as adjuvant	Phase I ¹³⁷
Epithelial cancer	Globo H–GM2–Lewis ^x –MUC1-32(aa)–sTn(c)–TF(c)–Tn(c)–KLH conjugate vaccine plus QS-21 as adjuvant	Phase I ¹⁴⁷
Melanoma	GM3NPhAc–KLH	Preclinical ¹⁵²
Prostate cancer	Unimolecular hexavalent conjugates (Globo H–GM2–Lewis ^x –sTn–TF–Tn–R)	Preclinical ¹⁴⁹
	TF(c)–KLH plus QS-21 as adjuvant	Phase I ¹³⁸
	Tn(c)–KLH and Tn(c)–palmitic acid	Phase I ¹³⁹
	Globo H–GM2–Lewis ^x –MUC1-32(aa)–TF(c)–Tn(c)–KLH conjugate vaccine plus QS-21 as adjuvant	Phase II ¹⁴⁸

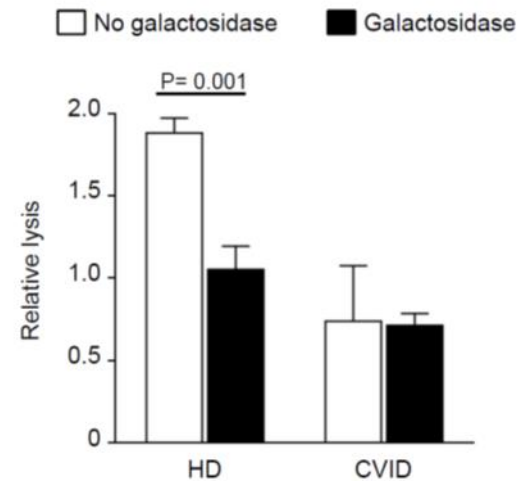
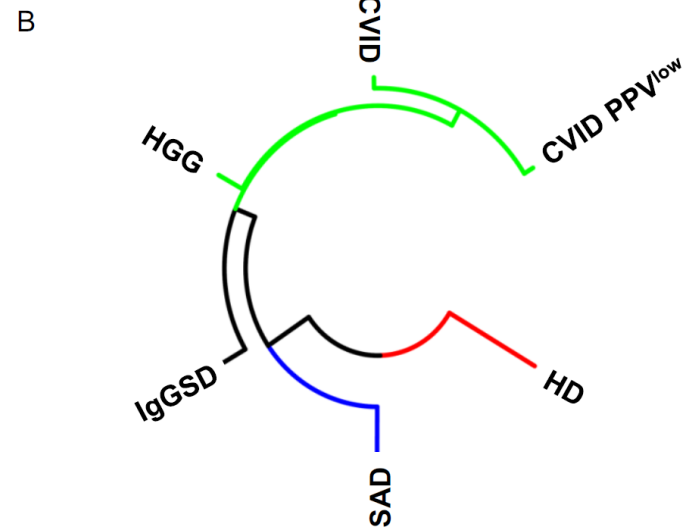
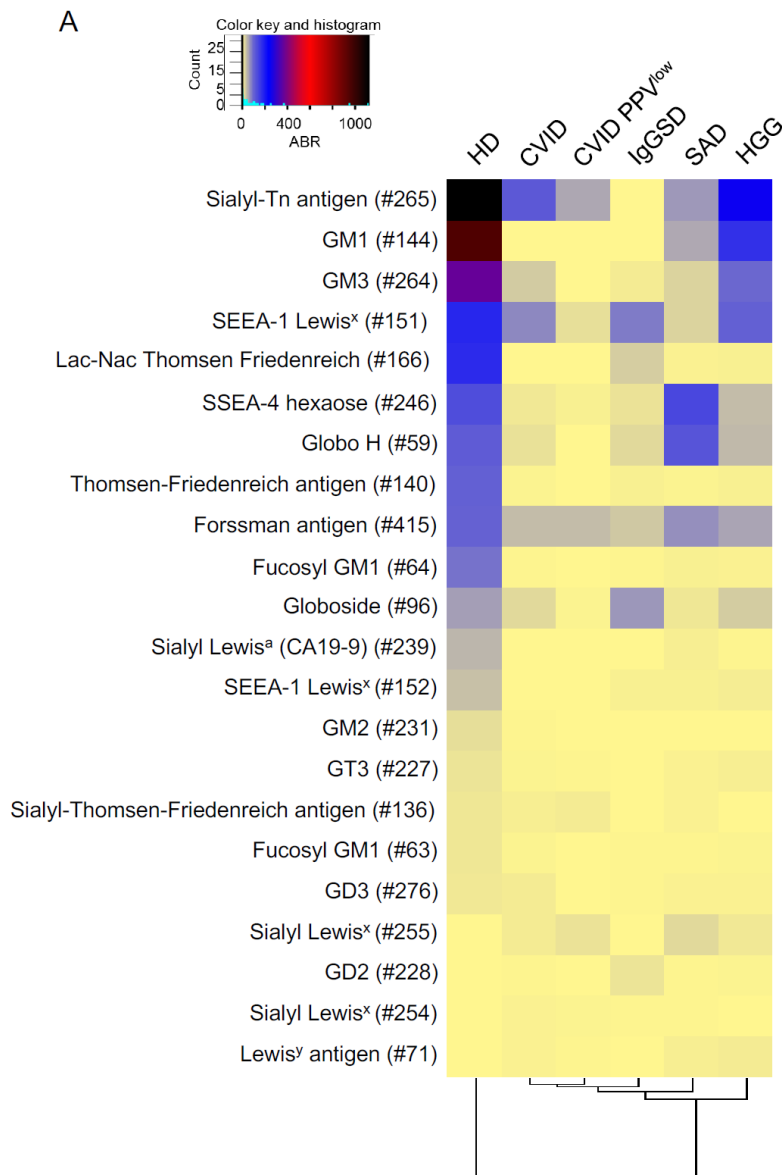
Cancer

Astronomo RD & Burton DR
Nat Rev Drug Discov 2016
 (modified)

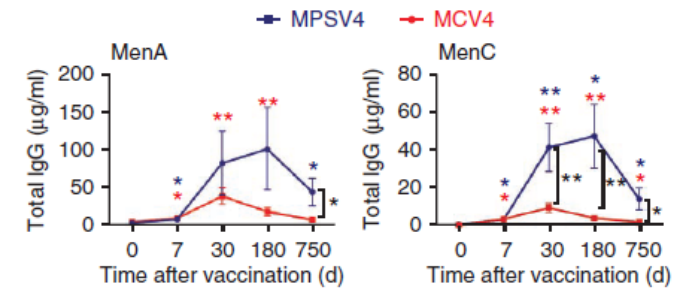
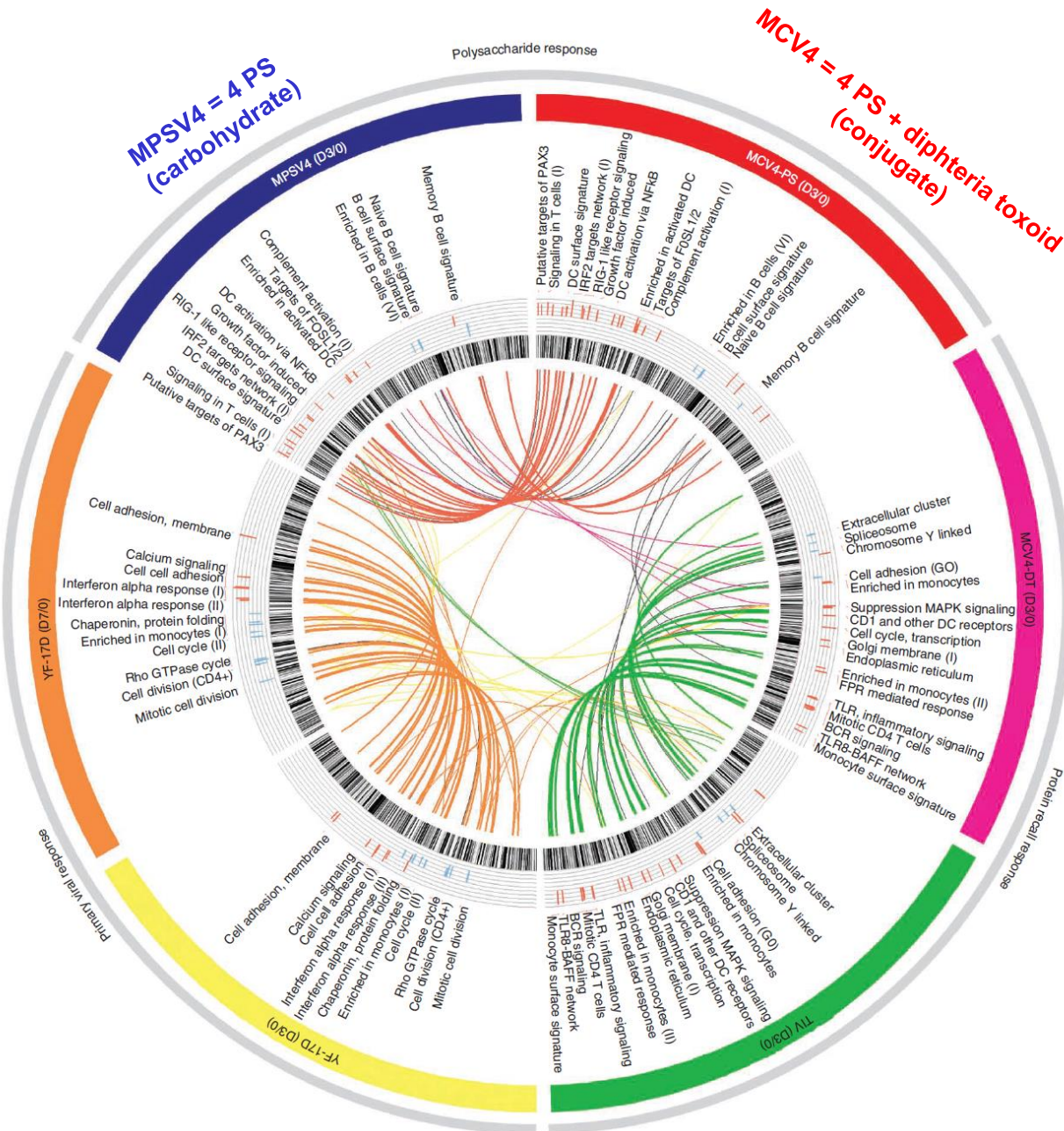
Table 2 Carbohydrate tumor markers

Marker ^a	Cancer type
AFP	Hepatocellular
β-hCG	Testicular, ovarian
CA 15-3	Breast, lung, prostate
CA 19-9	Gastrointestinal (pancreatic)
CA 27.29	Breast, lung, prostate
CA 125	Ovarian
CA 549	Ovarian
CEA	Colorectal
CEACAMs	Colorectal, pancreatic
HER2	Breast
onfFN	Thyroid
PLAP	Testicular, muscle
PSA	Prostate
sTn antigen	Colon, other
TAG-72	Ovarian, other
TG	Thyroid
Tn antigen	Colon, breast, cervical, other

Loss of Abs to tumor-associated carbohydrates (TACAs) in human primary antibody deficiencies



MENINGOCOCCAL VACCINATION

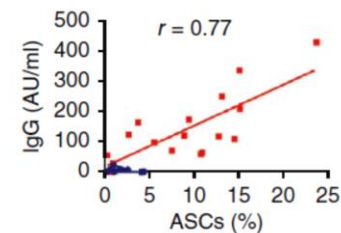
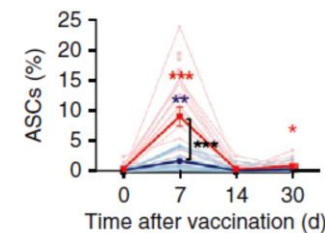


>25 yrs (USA)

MPSV4 = 4 PS
(carbohydrate)

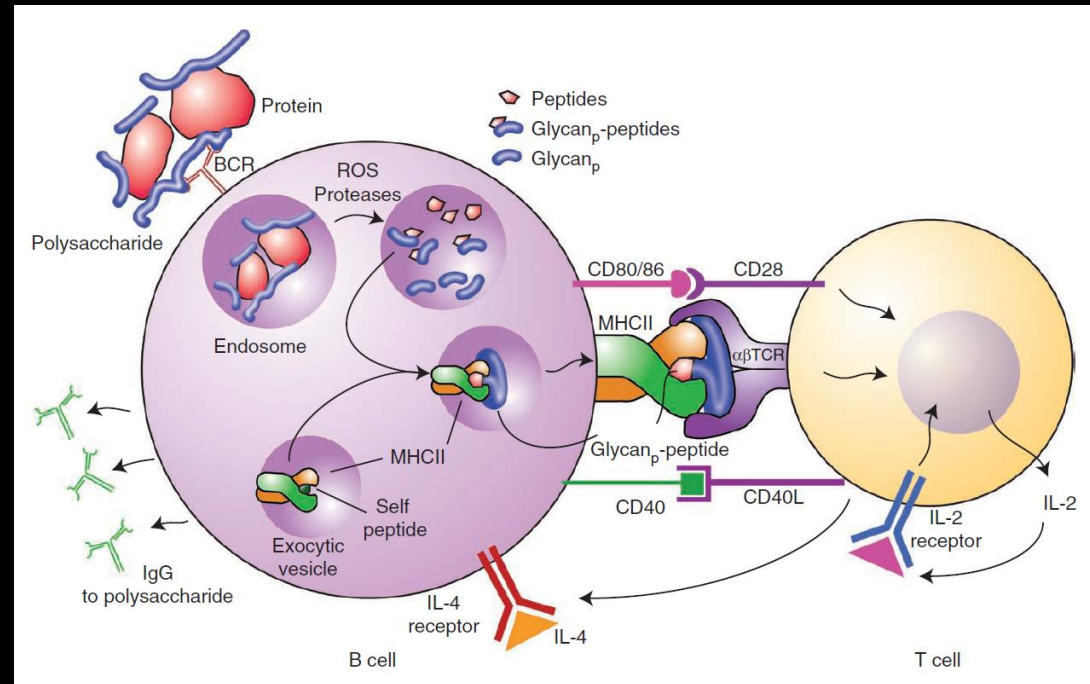
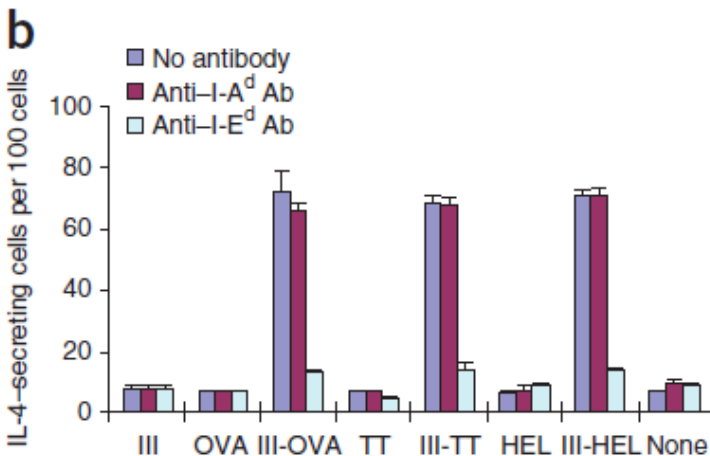
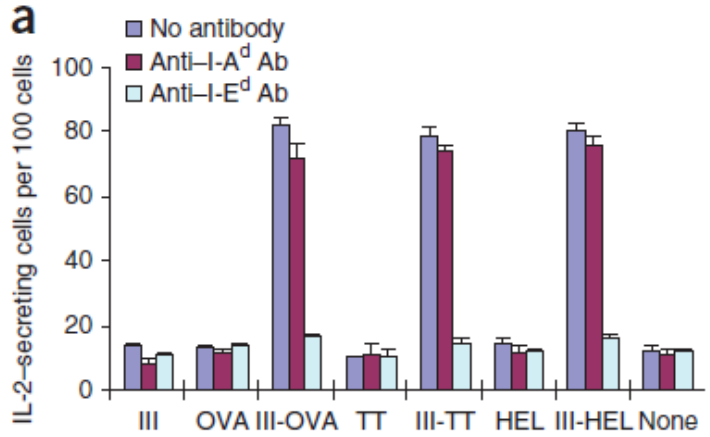
2005

MCV4 = 4 PS
+ diphtheria toxoid
(conjugate)



CONCEPTS ON GLYCAN-SPECIFIC ANTIBODIES: CARBOHYDRATE-SPECIFIC T-CELLS

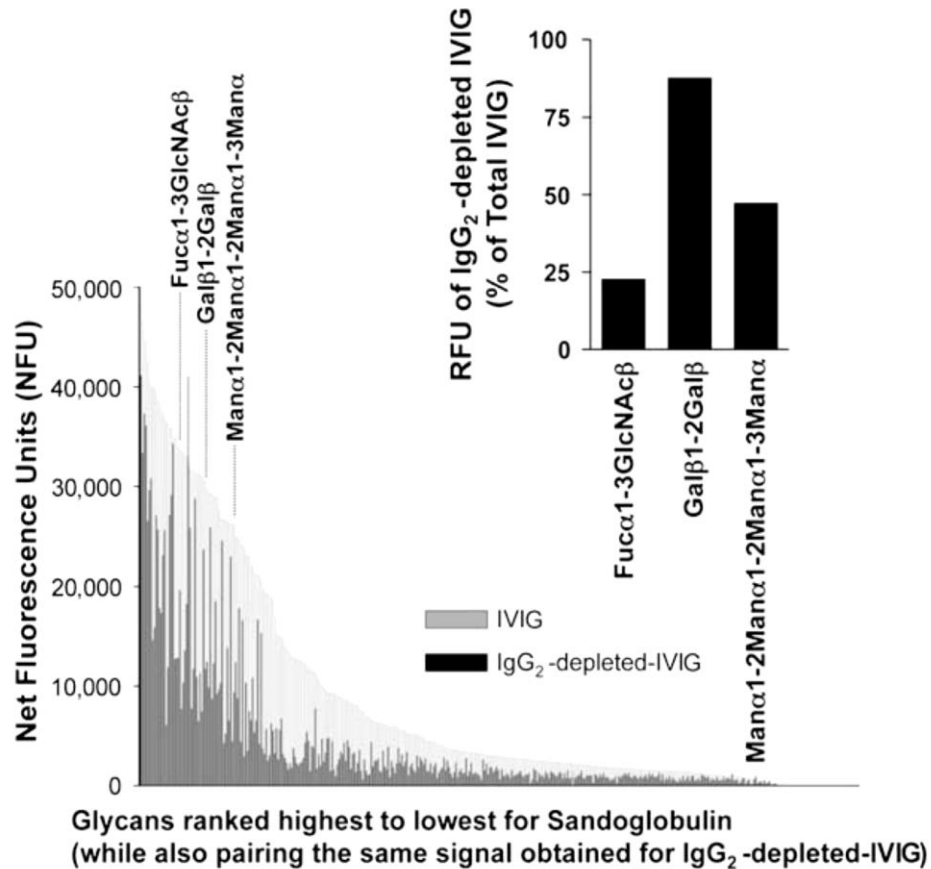
CD4⁺T cells, spleen, BALB/c mice



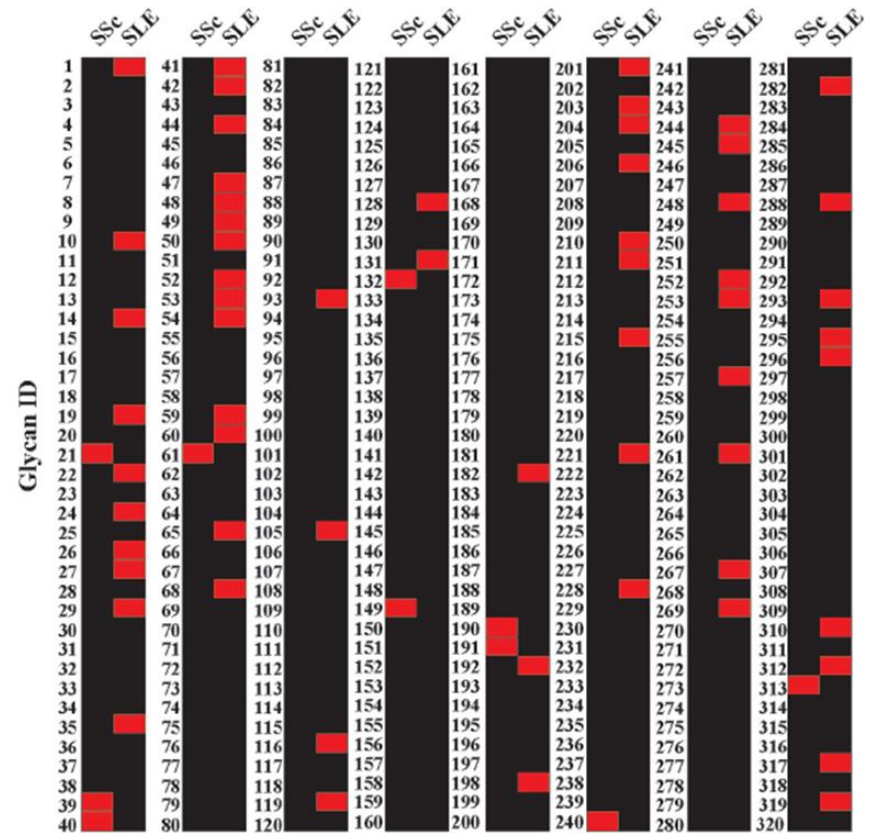
Avci FY et al. Nat Med 2011

III (type III polysacch. group B Streptoc.:GBSIII)
Carrier proteins: OVA, TT, HEL

GLYCAN ARRAY – PREVIOUS STUDIES

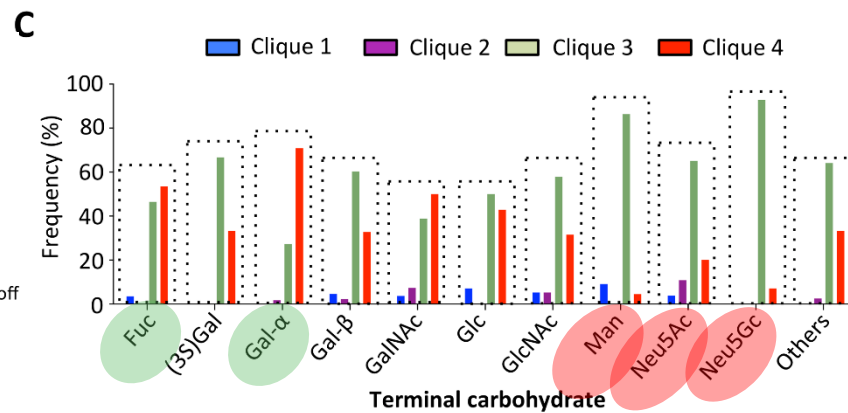
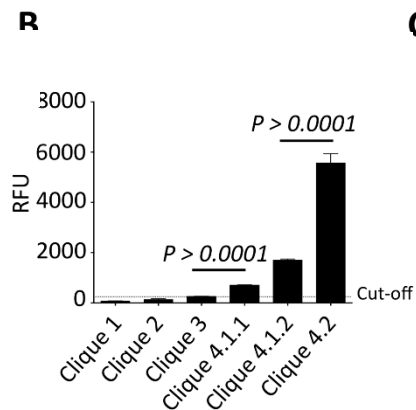
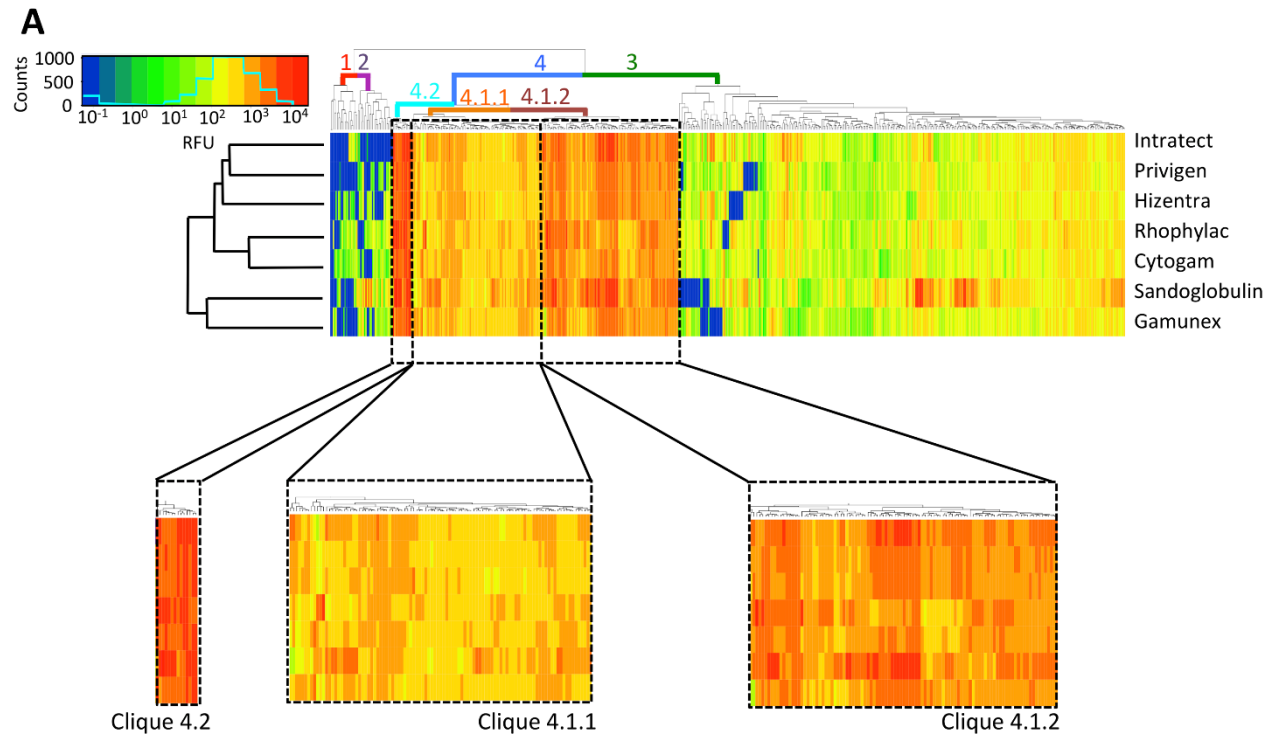


von Gunten S et al. J Allergy Clin Immunol 2009



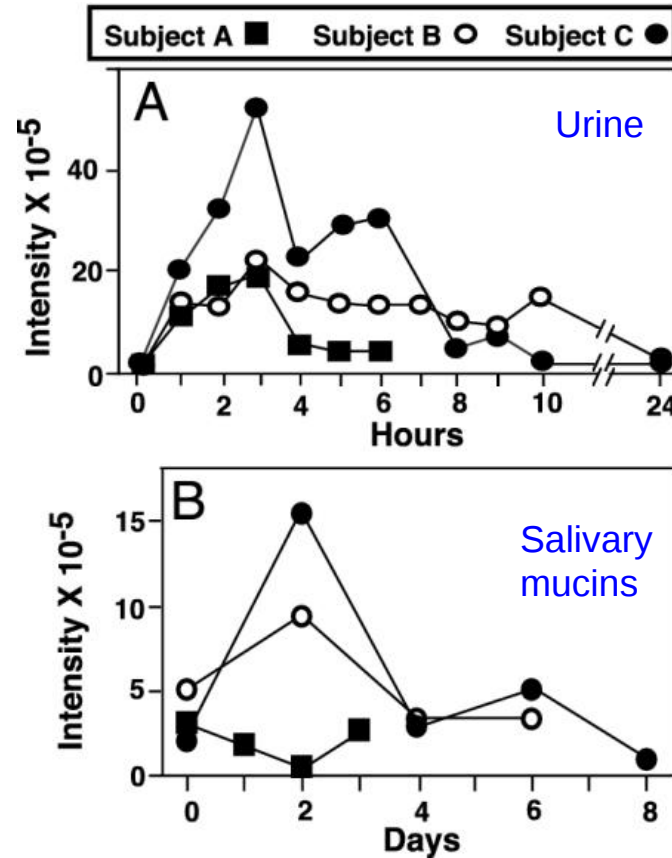
Grader-Beck T et al. Ann Rheum Dis. 2011

Antibody repertoire: glycan immunogenicity linked to structure



Dietary uptake and metabolic incorporation of non-human sialic acid

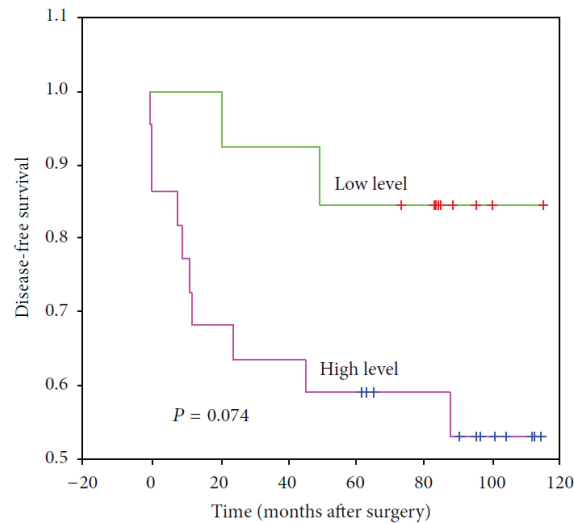
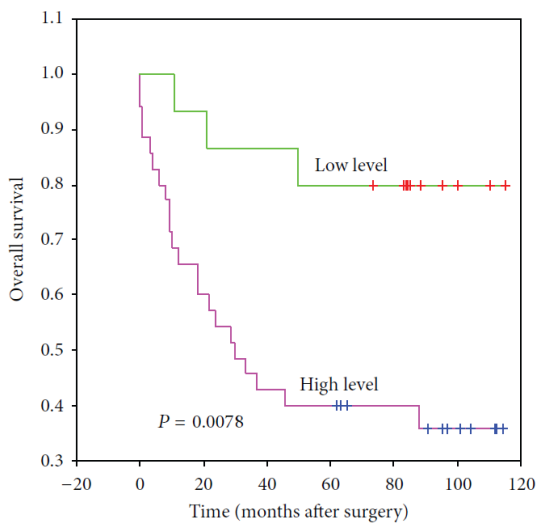
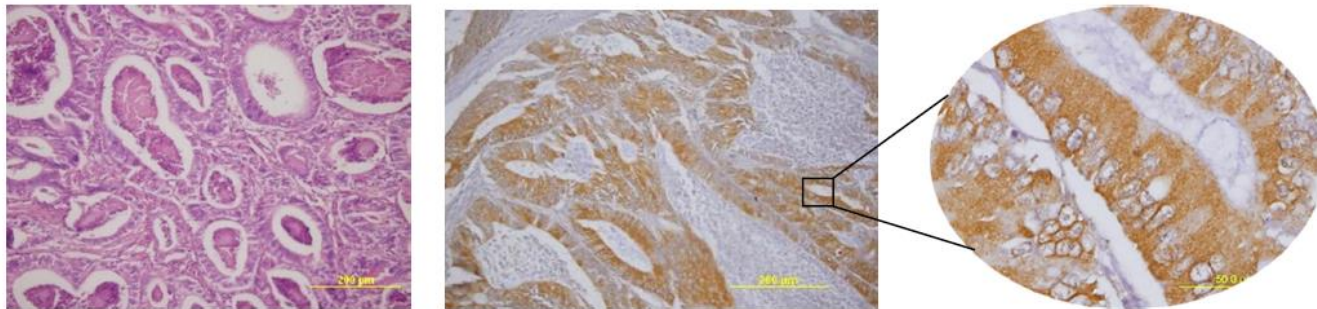
Neu5Gc uptake of ingested Neu5Gc



Tangvoranuntakul et al. PNAS 2003

Non-human sialic acid enriched in human tumors

GM3(Neu5Gc) in colon cancer

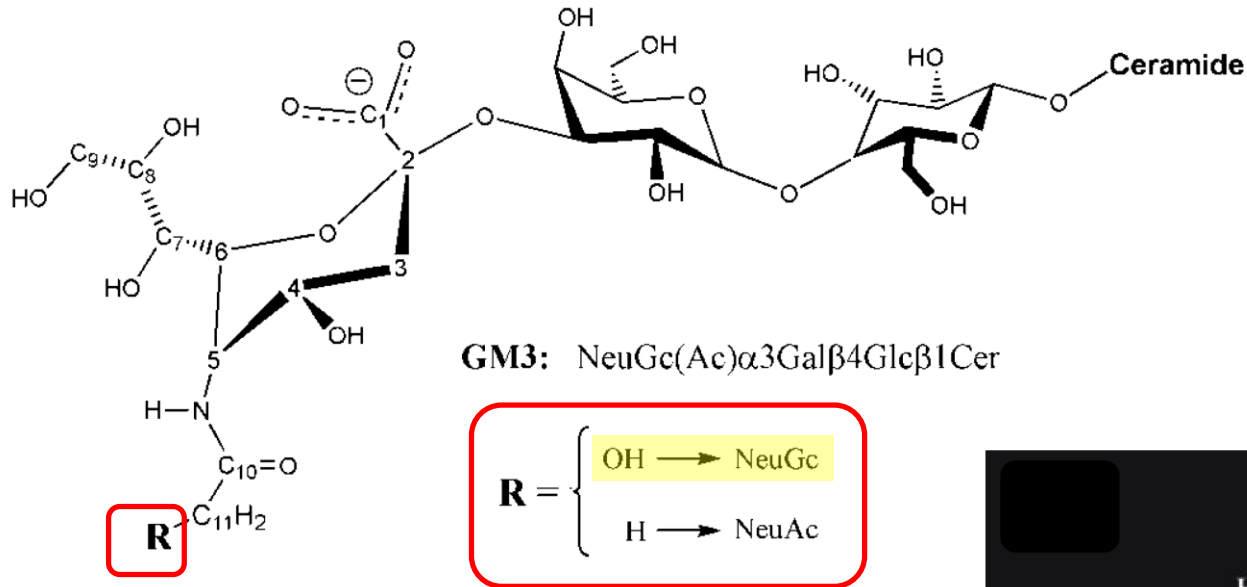


Lahera et al. J Oncol 2014

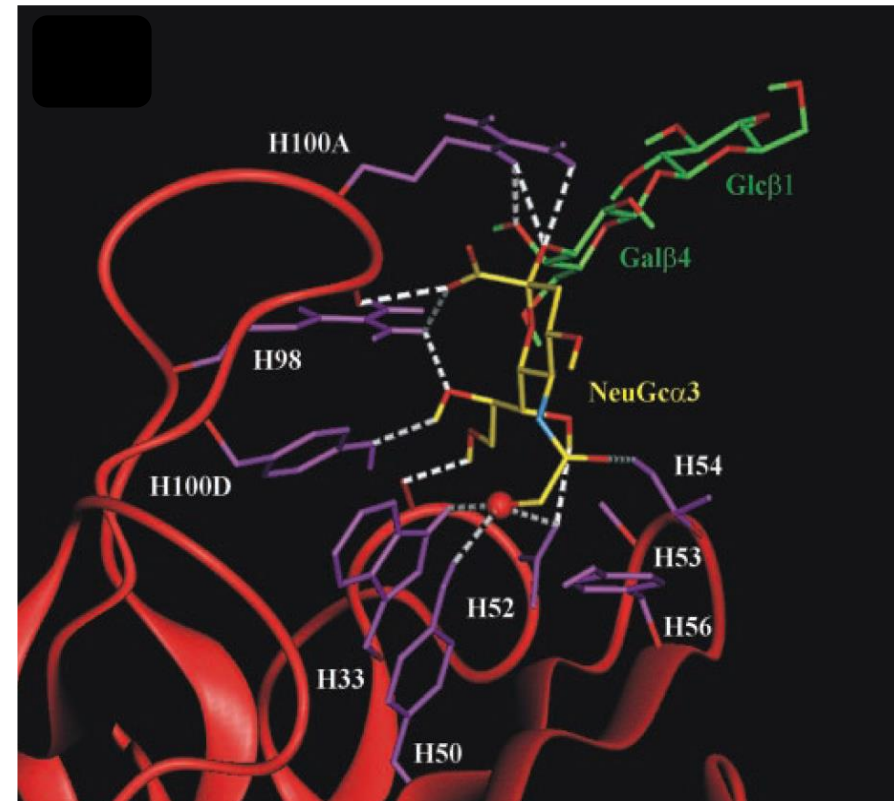


Olivia JP et al.
Breast Cancer Res Treat 2006

14F7 antibody against xenogeneic GM3(Neu5Gc)

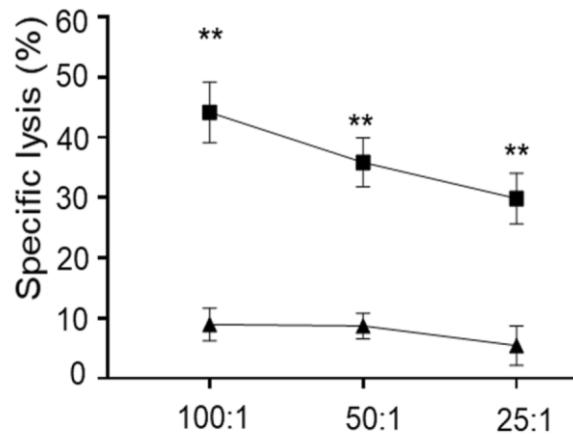
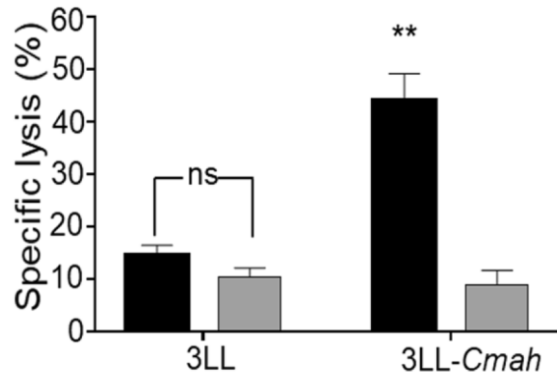


Krengel et al. J Biol Chem 2004

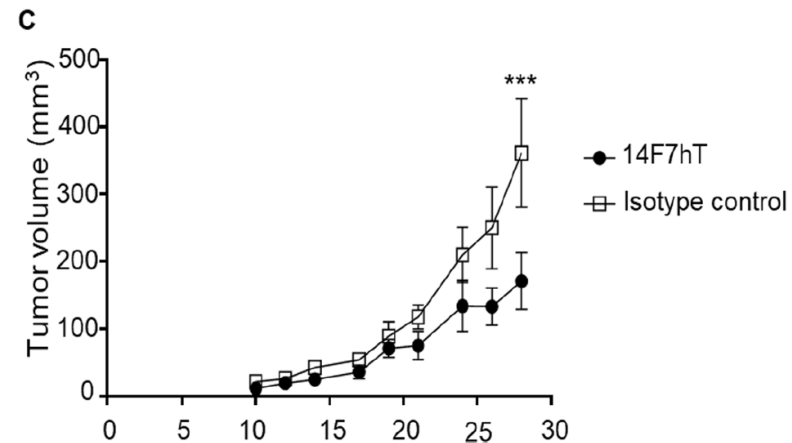
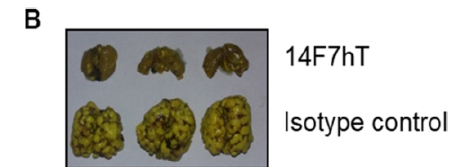
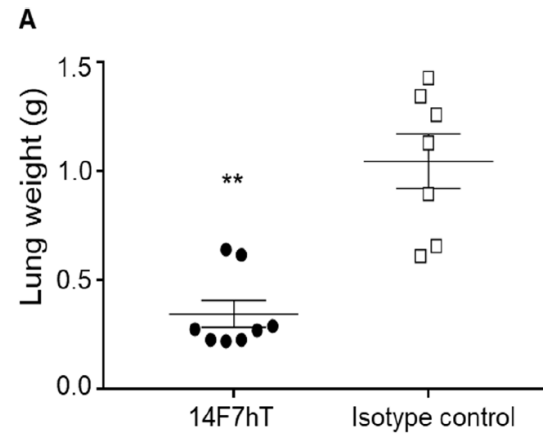


Non-human sialic acid enriched in human tumors

In vitro



In vivo



Collaboration with Center of Molecular Immunology (CMI), Havana

Dorvignit et al. Sci Rep 2019

Glycan involved	Proposed major function(s)	Possible therapeutic targeting	Examples of neoplasms	References
Growth and proliferation				
<i>N</i> -glycans	Suppress apoptosis; growth-factor signalling	Alkaloid inhibitors of <i>N</i> -linked processing	Breast, melanoma, Ewing's sarcoma	16,18
<i>O</i> -glycans	Mucin (MUC4)-mediated activation of ERBB2 receptors	Immunotherapy targeting MUC4 (similar to other mucin-targeting immunotherapy)	Breast	18
<i>O</i> -glycans	Suppress apoptosis (possibly through galectin-3 binding to tumour <i>O</i> -glycans expressing terminal galactose)	Galectin-3 inhibitors (β -galactosides)	Colon, pancreatic	150
Glycosphingolipids	Control of signalling through lipid rafts	Ceramide glycosylation inhibitors; ganglioside-targeted vaccines	Breast	20
Heparan-sulphate proteoglycans	Coreceptors for tumour growth factors	Heparin derivatives as heparan-sulphate competitors; sulphotransferase inhibitors	Pancreatic, ovarian, renal, hepatic	24,25
Hyaluronan	Signaling through hyaluronan receptors (for example, CD44)	Hyaluronan oligomers; adenoviral delivery of hyaluronan-binding protein genes	Colon, breast	13,30
<i>O</i> -GlcNAc	Modify oncogene phosphorylation	<i>O</i> -GlcNAc transferase inhibitors	Pancreatic	31
Tumour invasion				
<i>N</i> -glycans	Alter E-cadherin-dependent tumour adhesion	Alkaloid inhibitors of <i>N</i> -glycan processing	Breast, colon	41,74
<i>N</i> -glycans	Tumour repulsion (for example, polysialylation)	Sialyltransferase inhibitors	Neuroblastoma, lung (small cell)	43
<i>O</i> -glycans	Stimulate migration; potentiate migration of tumour cells through inhibition of cell-cell contacts (for example, sialyl Tn on mucins)	Vaccines (for example, conjugated sialyl Tn)	Breast, gastric, ovarian	47
Glycosphingolipids	Tumour repulsion (for example, G_{M2})	Glycosphingolipid inhibitors; ganglioside-targeted vaccines	Melanoma, neuroblastoma, breast	35,36, 134,151
Heparan-sulphate proteoglycans	Matrix growth factor storage (heparanase substrate)	Heparin fragments and analogues; sulphotransferase inhibitors; xylosides; antisense RNA to perlecan	Breast, colon, hepatic, lymphoma, melanoma	68,69,72
Chondroitin-sulphate proteoglycans	Modulate tumour-matrix attachment	Xylosides	Melanoma, glioma, lung	61-63
Hyaluronan	Coordinate tumour growth signalling with cytoskeletal events during migration	Target tumour hyaluronan receptors (for example, gene silencing of CD44)	Breast	28
Tumour metastasis				
<i>O</i> -glycans	Facilitate tumour adhesion during haematogenous metastasis (SLe ^x , SLe ^a and other selectin ligands);	Disaccharide primers of glycosylation (reduce tumour SLeX); competition by intravenous heparin	Colon	101,112,118
<i>N</i> -linked and <i>O</i> -linked glycans	Promote tumour aggregation (galectin-3 binding)	Galectin-3 inhibitors (β -galactosides)	Melanoma	150
Glycosphingolipids	Tumour adhesion (sulphated selectin ligands)	Disaccharide primers; competition with heparin	Colon	101,118
Tumour angiogenesis				
<i>N</i> -glycans	Promote migration of endothelia	Alkaloid inhibitors of <i>N</i> -linked glycosylation	Prostate	152
Heparan-sulphate proteoglycans	Co-receptor for growth factors; matrix growth factor storage; co-receptor for matrix proteins	Heparin fragments and analogues; sulphotransferase inhibitors; xylosides; antisense RNA to perlecan	Colon, renal, melanoma, breast	9,71
Tumour immunity				
Glycosphingolipids	Immune 'silencing' (ganglioside shedding)	Ganglioside vaccines	Melanoma, neuroblastoma, breast	35,36,134

Tumor biology:

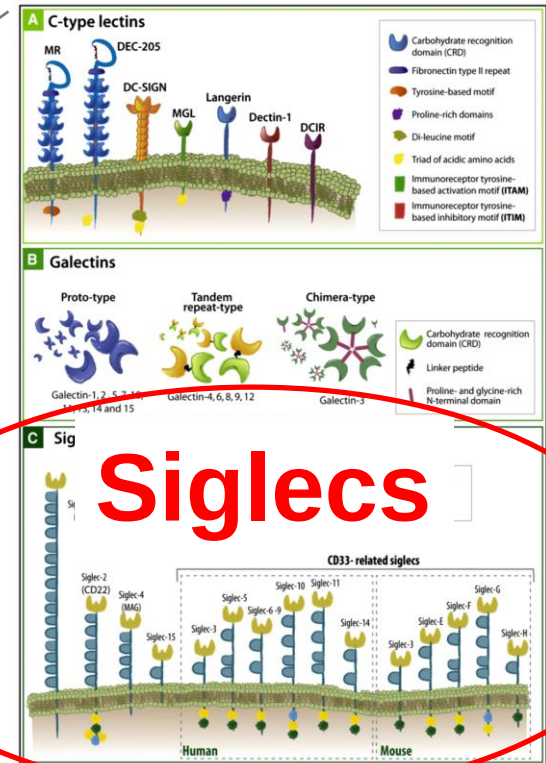
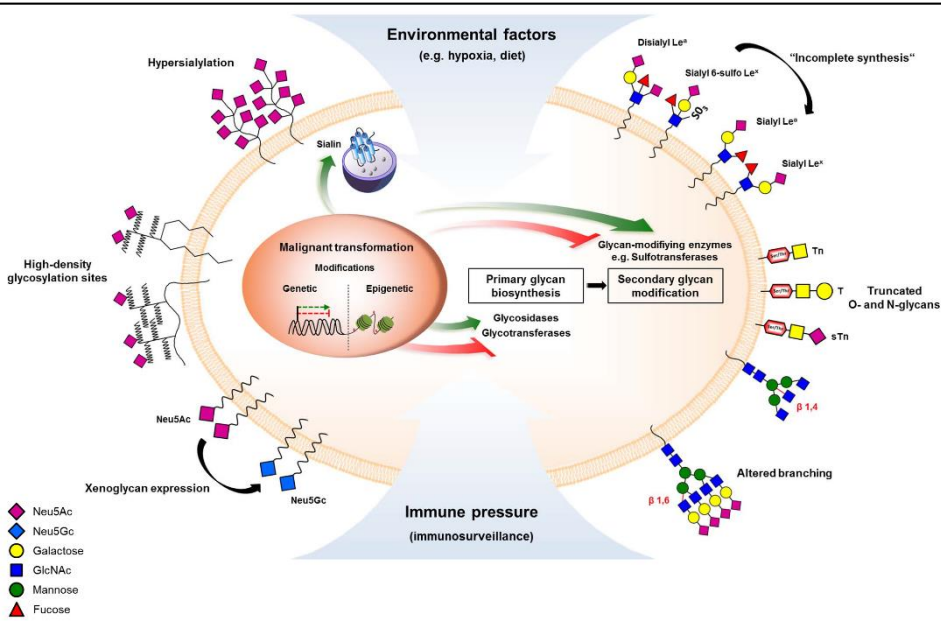
Multifaceted role of sugar structures

**Fuster MM & Esko JD,
Nat Rev Cancer 2005**

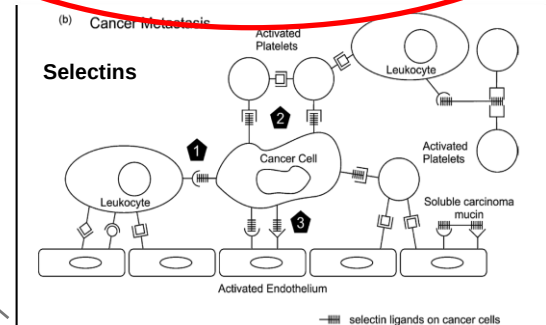
Deciphering the glycan code

Sensor system (lectins,...)

Glycan code



Siglecs



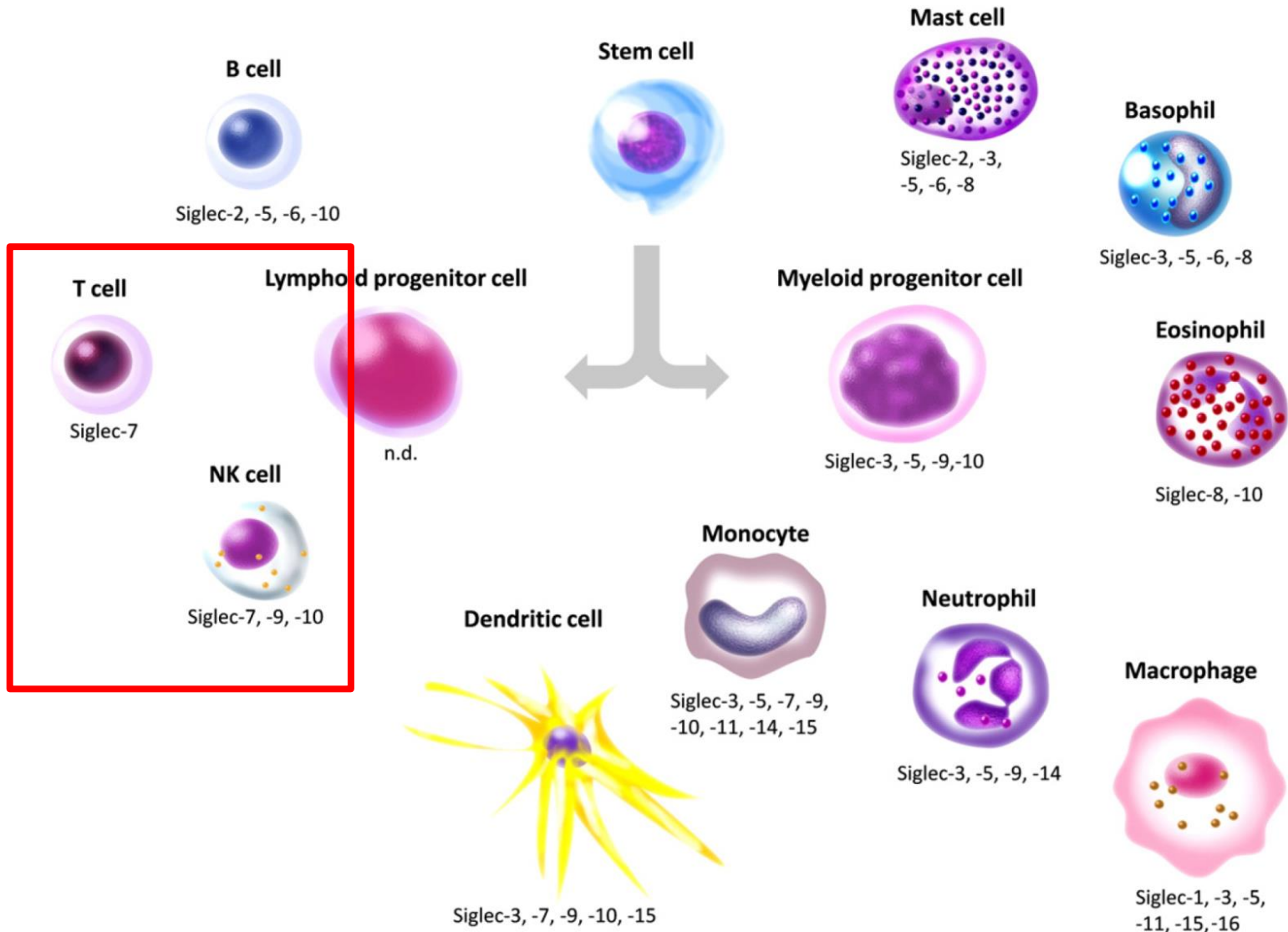
Cancer intelligence acquired (CIA): tumor glycosylation and sialylation codes dismantling antitumor defense

Boligan KF et al. Cell Mol Life Sci, 2015

Rabinovich GA & Croci DO, Immunity 2012

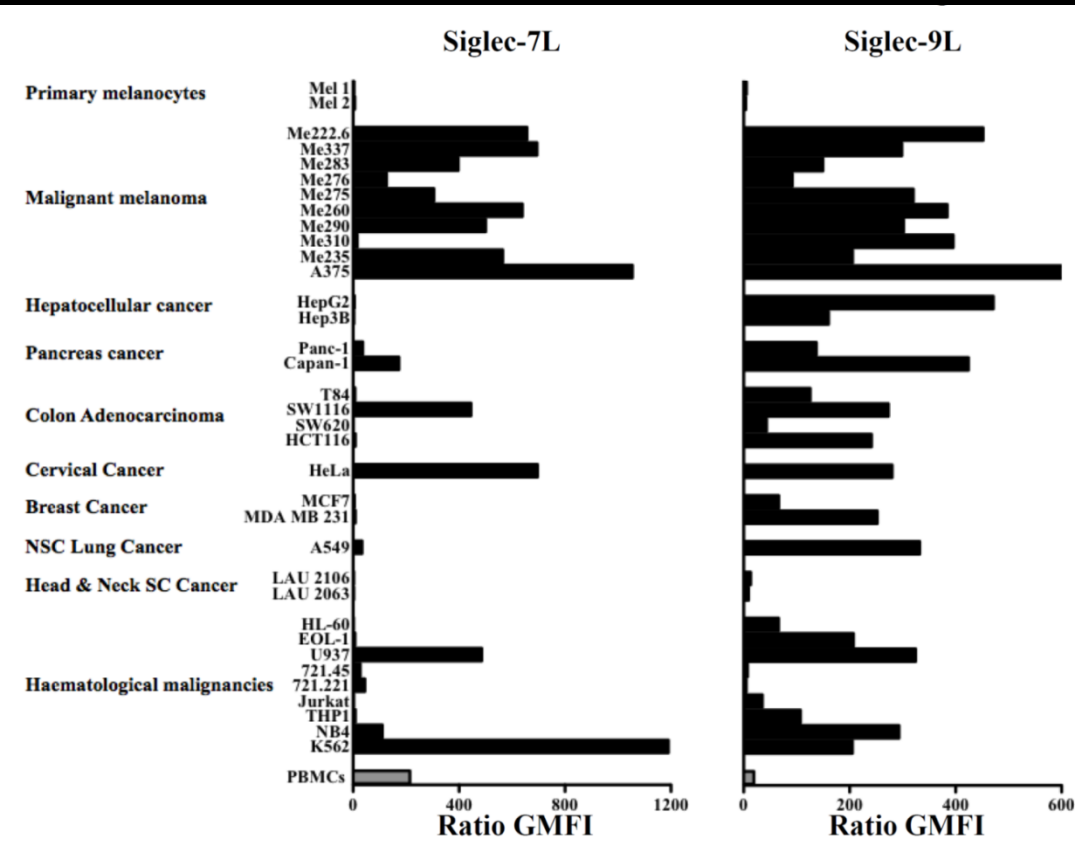
Läubli H & Borsig L, Sem Cancer Biol 2010

Siglec expression on immune cells

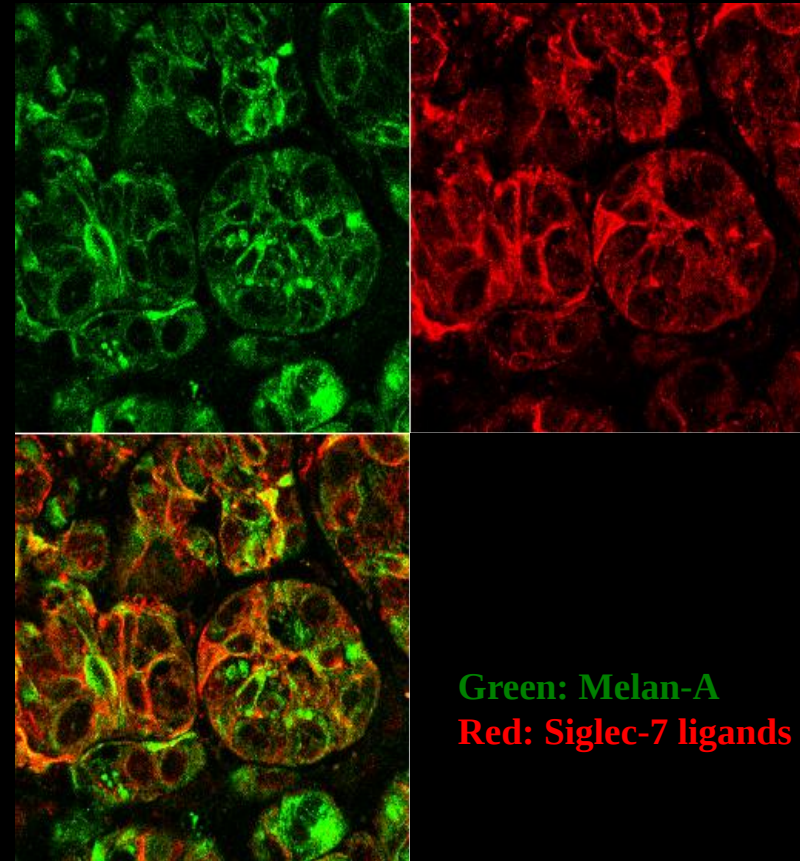


Siglec-7/-9 Ligand expression on tumor cells

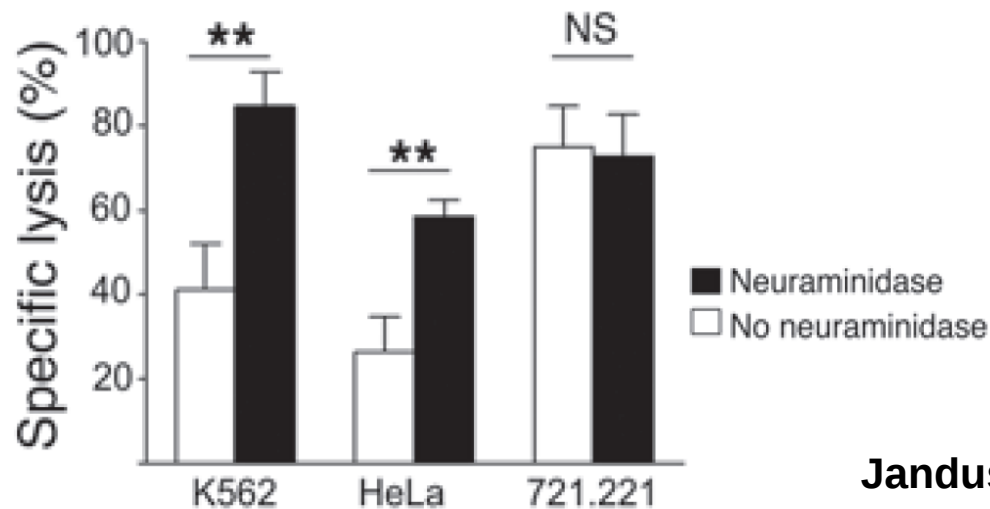
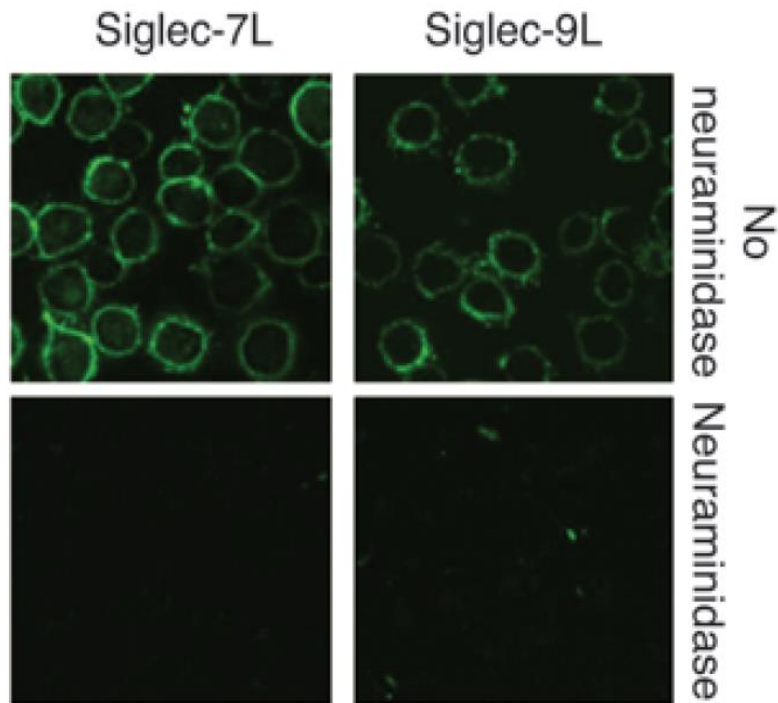
Flow cytometry: Siglec-7 and -9 ligands on cell lines



Tissue section: Siglec-7 Ligands in melanoma

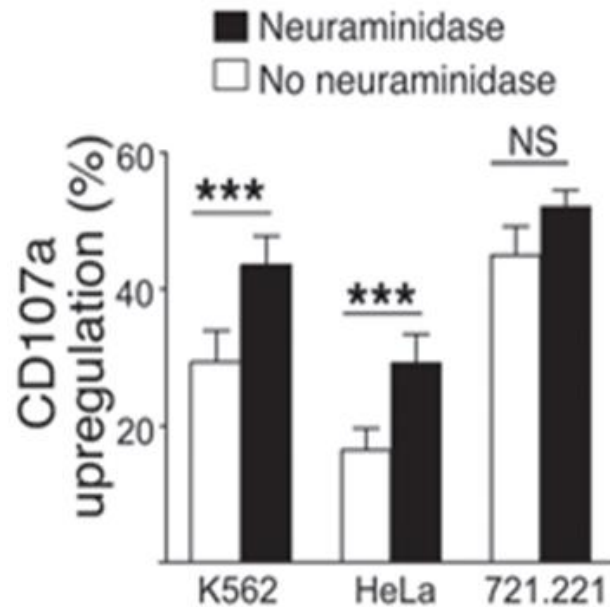


The sialoglycan shield

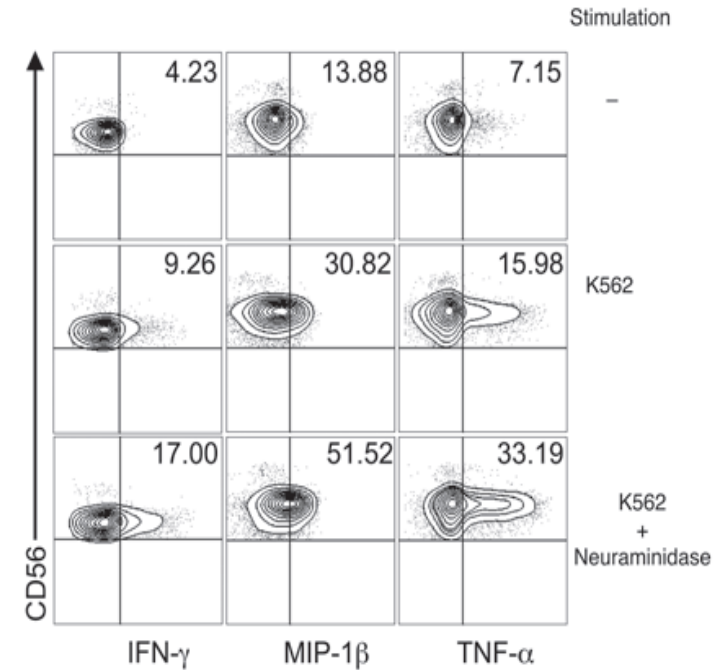


Desialylation of target cells enhances NK cell antitumor activities

Degranulation

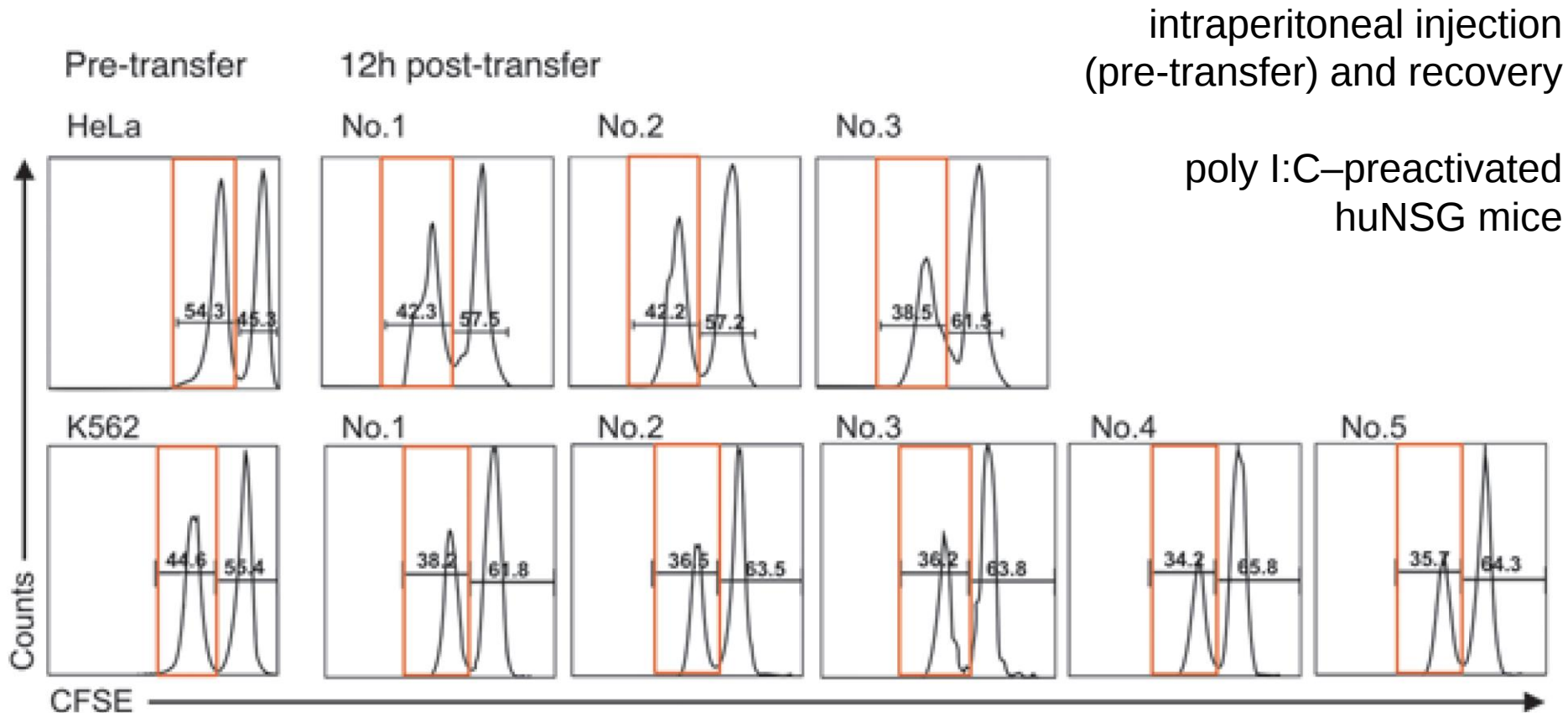


Cytokine production



Humanized mice with reconstituted human Siglec-7⁺ NK cells

Accelerated elimination of desialylated K562 and HeLa cells *in vivo*





James P Allison
MD Anderson Cancer Center



Tasuku Honjo
Kyoto University



Siglecs on CD8 T cells?

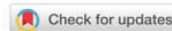
May 2019 Cover Story American Association of Cancer Research (AACR)

Research Articles

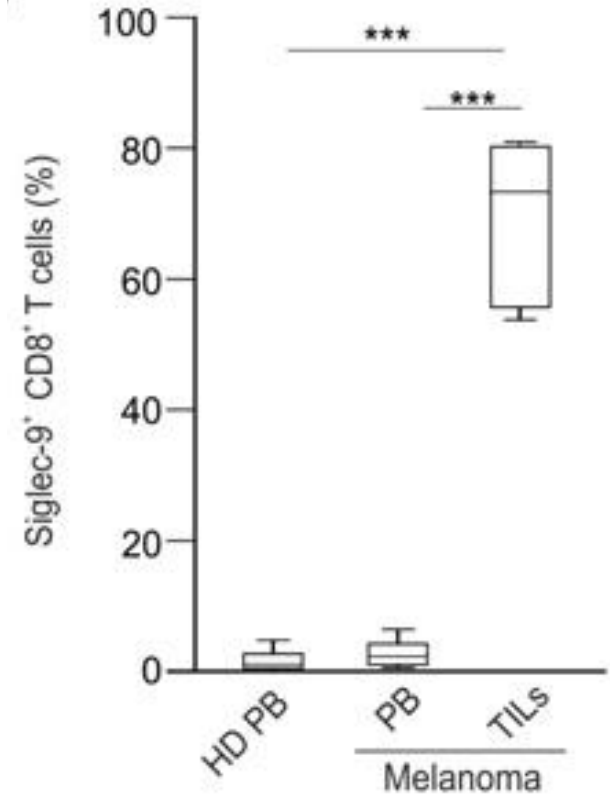
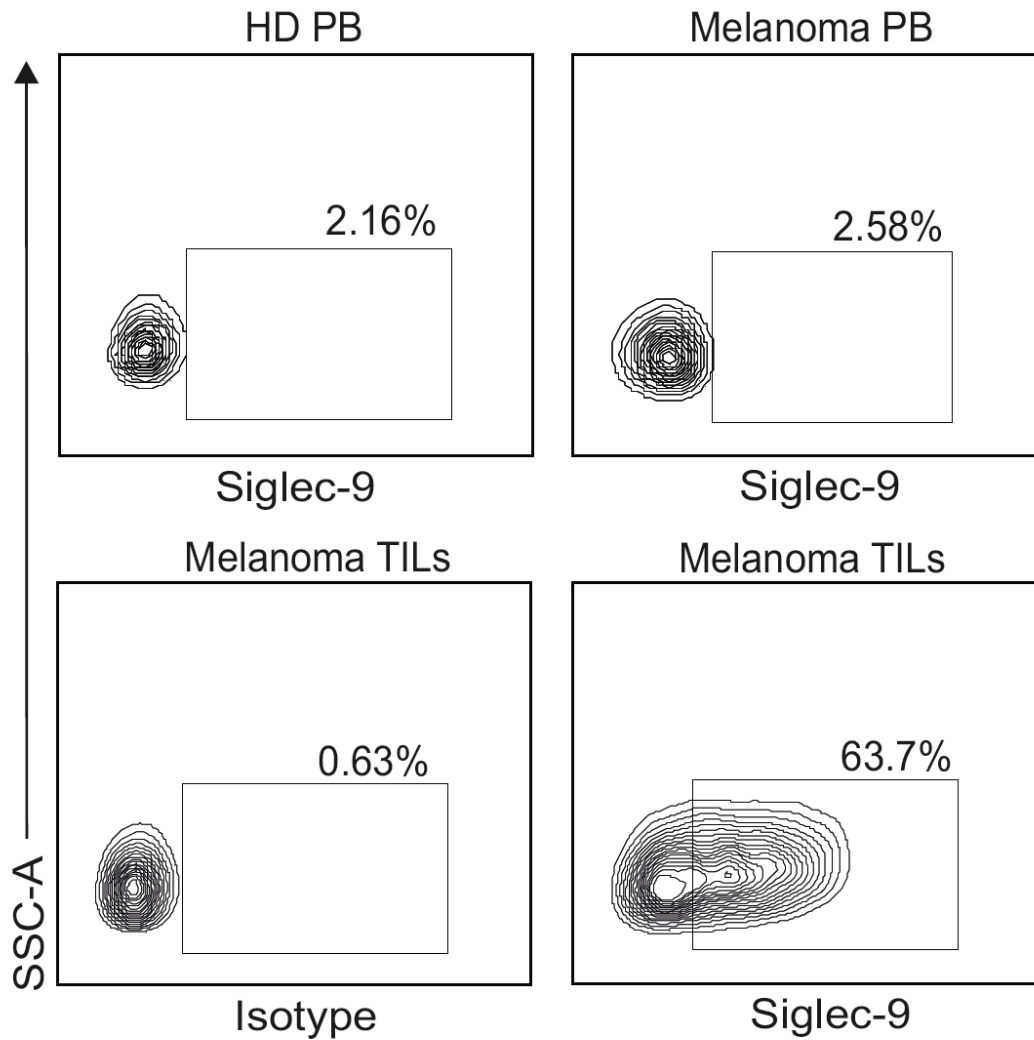
Siglec-9 Regulates an Effector Memory CD8⁺ T-cell Subset That Congregates in the Melanoma Tumor Microenvironment

Quentin Haas, Kayluz Frias Boligan, Camilla Jandus, Christoph Schneider, Cedric Simillion, Michal A. Stanczak, Monika Haubitz, Seyed Morteza Seyed Jafari, Alfred Zippelius, Gabriela M. Baerlocher, Heinz Läubli, Robert E. Hunger, Pedro Romero, Hans-Uwe Simon, and Stephan von Gunten

DOI: 10.1158/2326-6066.CIR-18-0505 Published May 2019

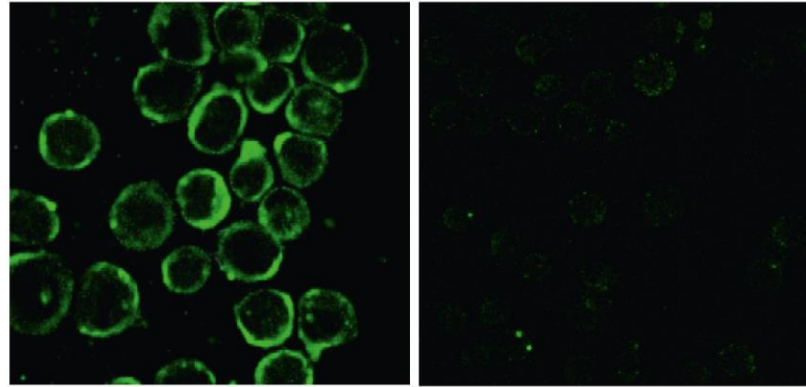


Siglec-9⁺ CD8 T cells in melanoma



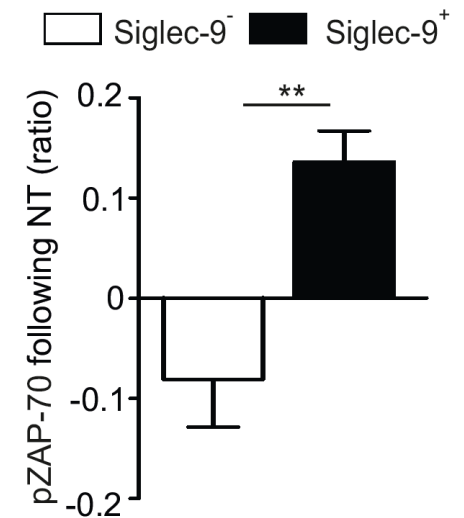
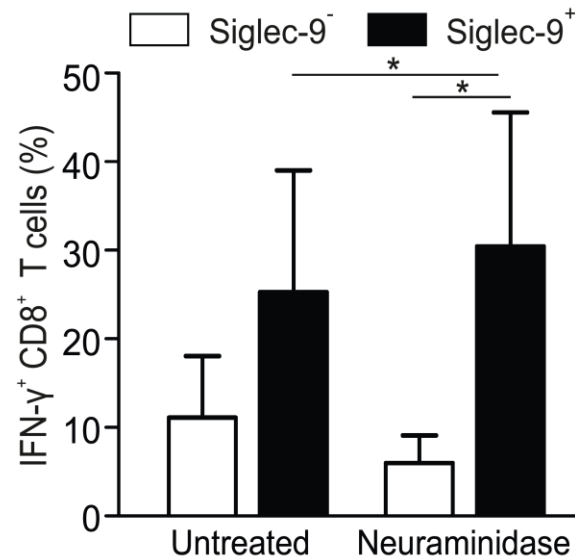
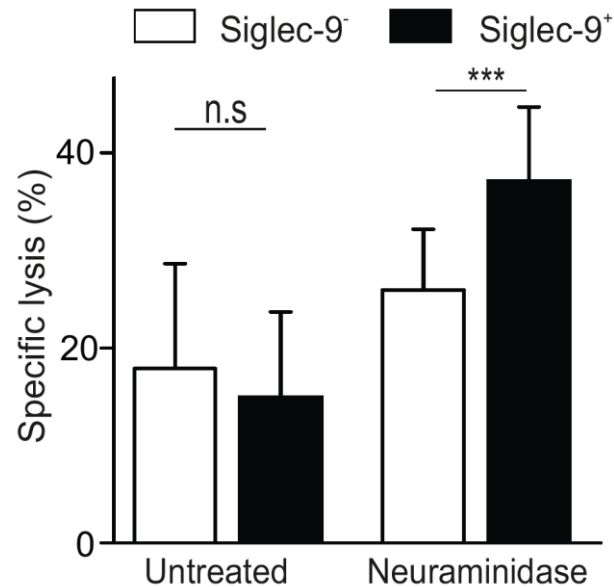
Glycosylation as immune checkpoint for Siglec-9⁺ cytotoxic T cells

P815 target cells

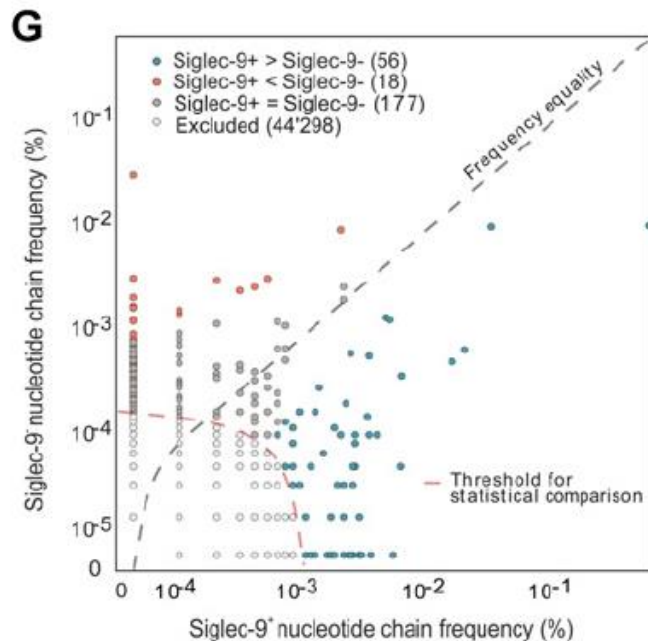
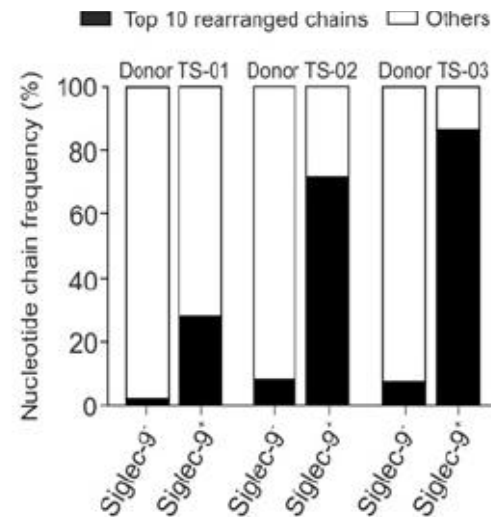
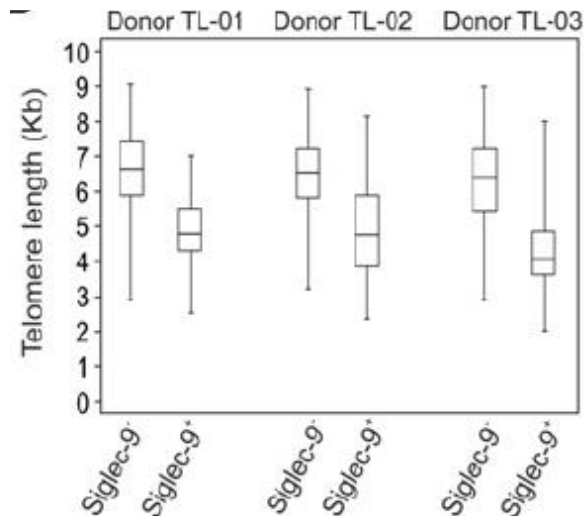


No neuraminidase

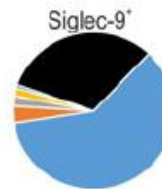
Neuraminidase



Siglec-9⁺ CD8 T cell undergo clonal expansion



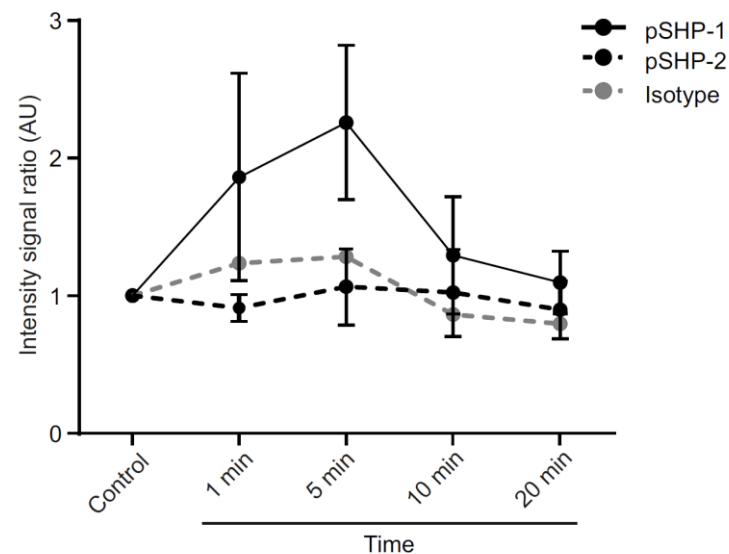
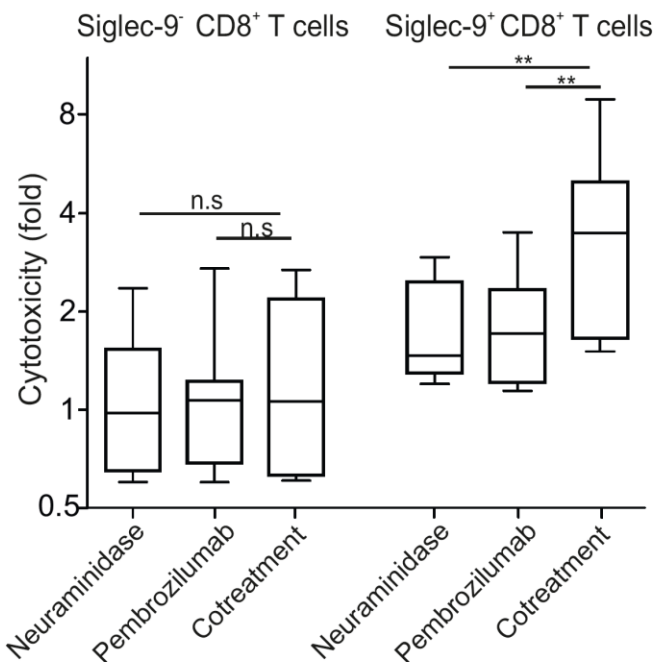
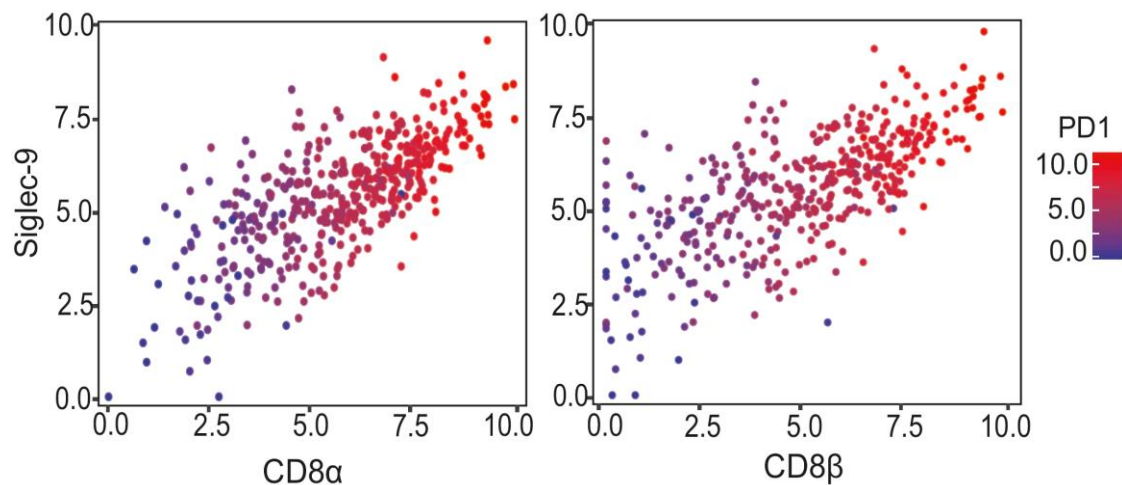
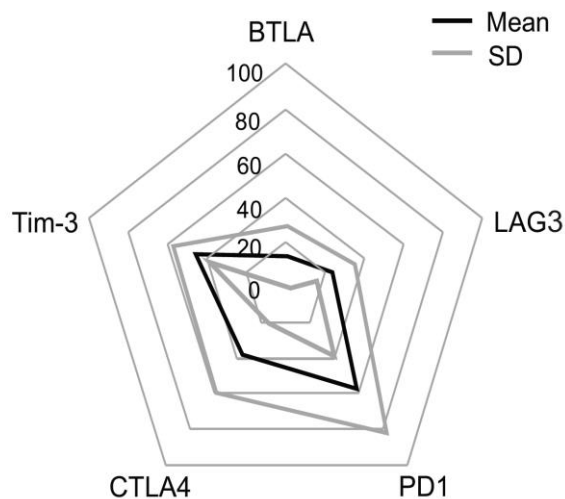
H



CCTGAAGACAGCAGCATAATCTCTGGG...	1	CTTTAGCTCCTACATGAGCAGTTCTTCGGGCCA	60.66%
AATGTGAGCACCCTTGGAGCTGGGGGACTC...	1	AGTGGAGGGGATCAGCCCGACATTTTGGTGAT	3.44%
GGGTGGAGTCGGCTGCTCTCCCTCCCAATL...	1	TACTCAGGGAACACTGAAGCTTCTTTGGACAA	2.10%
ACGTTGGCGTCTGCTGTACCTCTCAAGL...	1	TGGGGGAACACCGGGGAGCTGTTTTTGGAGAA	1.68%
CTGATTCGGAGTCGGCCAGCAGCAACACCT...	1	AGTTTGTTCGGTCAAGCCAGCATTTCGGTGAT	0.86%
GAGATCCAGCGCACAGCAGAGGGGGACTL...	1	CGGCCAGGGGAACTGAAGCTTCTTTGGACAA	0.65%
GGCTCTGCTGTACCTCTCAGACATCTGTG...	1	TAGCGAACACCGGGAGCTGTTTTTGGAGAA	0.57%
GCAMAGCTTGAGGACTCGCCCTGTATCTL...	1	GGGCTTCTGGAGGGGCTACACCTTCGGTTG	0.53%
CTGAAGGTGACGCTGCAGAACTGGAGGAL...	1	AGCCCGGACAGGAGCGGCTACACCTTCGGTTG	0.50%
GGCTCTGCTGATCCCTCTCAGACATCTGTG...	1	GGGGCTCCTACATGAGCAGTTCTTCGGGCCA	0.42%

CTGACAGTGACAGTGCCCATCTGAAGAL...	1	AGGAGGGTGAACACTGAAGCTTCTTTGGACAA	3.15%
AATGTGAGCACCCTTGGAGCTGGGGGACTC...	1	TGGAGGGGATCAGCCCGACATTTTGGTGAT	1.02%
CCTGAAGACAGCAGCATAATCTCTGCAGL...	1	TTTAGCTCCTACATGAGCAGTTCTTCGGGCCA	1.01%
GTGAGCACCCTTGGAGCTGGGGGACTCGGG...	1	GGACAGACACAGATACCGCAGATTTTGGCCCA	0.93%
GAGTCGCCAGCCCAAGCAGACCTCTCTL...	1	AGCATCTACATGAGCAGTTCTTCGGGCCA	0.63%
CTGGAGTCGCCAGCAGCAACCAAGACATCT...	1	CAGCGGATTCCTACGACAGTACTTCGGGCCG	0.32%
CAAGCTCAGAACTGGAAGATTCGCAATL...	1	GGCCCTCGGGGCTGAAGCTTCTTTGGACAA	0.30%
CTGAGCTCTCTGAGCTCGGGGACTCAGCT...	1	GGGACAGCAGCACTGAAGCTTCTTTGGACAA	0.27%
CTG3AGTCGCCAGGCCCAACAGAGCTCTL...	1	GGGTGGCTCCTACGAGCAGTACTTCGGGCCG	0.26%
GACATCCGCTCACCAGGCTGGGGGACGCT...	1	AGAGTGGCGGGAGAGACCACTACTTCGGGCCA	0.24%

Siglec-9⁺ CD8 T cells as targets for immunotherapy in melanoma



A Cartography of Siglecs and Sialyltransferases in Gynecologic Malignancies: Is There a Road Towards a Sweet Future?



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