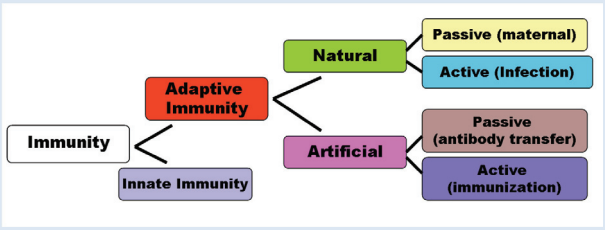
Immunity (defence – protection)

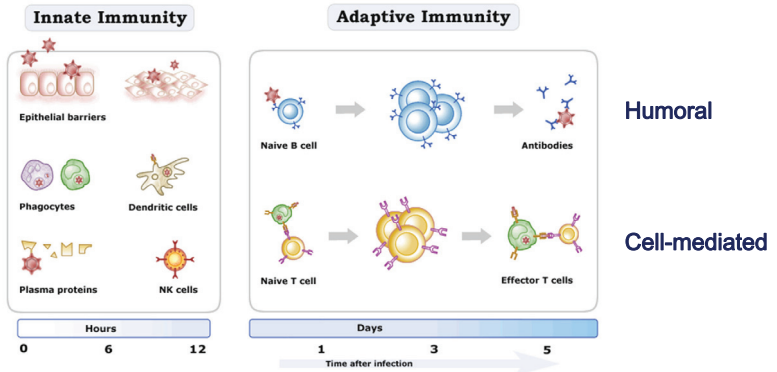
the resistance against diseases, microbes harmful species and in particular the capability to avoid infections

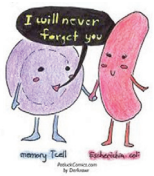
The ensamble of cells, tissues and molecules which mediate such resistance is called **Immune System**

Innate Immunity, is nonspecific immunity, is the natural resistances with which a person is born.
It provides resistances through several physical, chemical and cellular approaches.



Adaptive Immunity, sub-divided into two major types:
Naturally acquired immunity occurs through not deliberate contact with a disease causing agent
Artificially acquired immunity develops only through deliberate actions such as vaccination.





Specificity (for antigens structurally non-self – Self antigens are safe)
Immunologic memory (remember an ecounter with a antigen)

Immune System

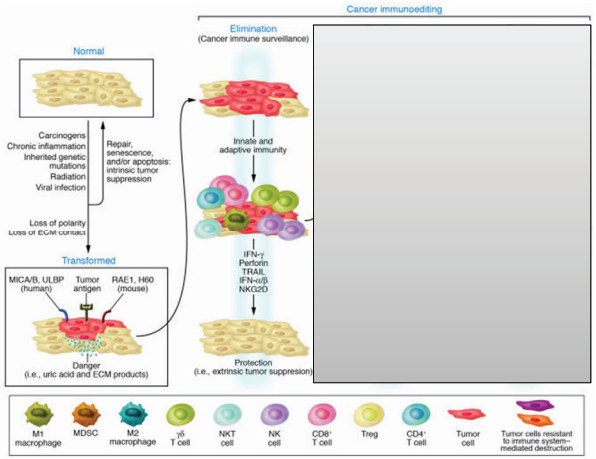
Immunity — **Protection** / **Surveillance**

Immuno-response *vs.* Tumors & Transplantation

Specifically identify and eliminate tumor cells on the basis of their TSA or TAA (celluar stress of harmful chemicals)

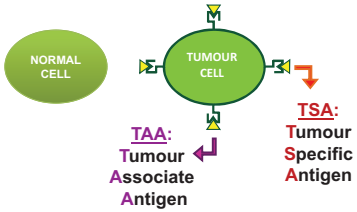
"Self or Non Self"
It makes the difference

Tumor & Immune System

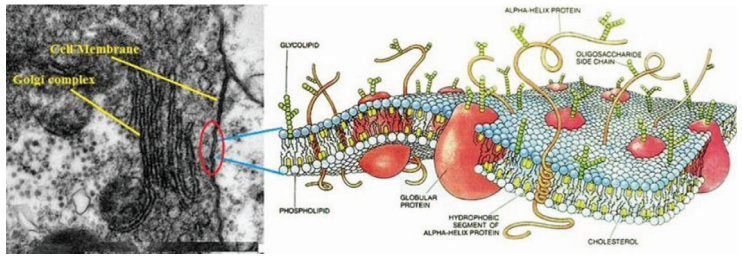


J.B. Swann & M.J. Smyth *The Journal Clin Invest*. **2007**, *117*, 1137

Tumor & Immune System

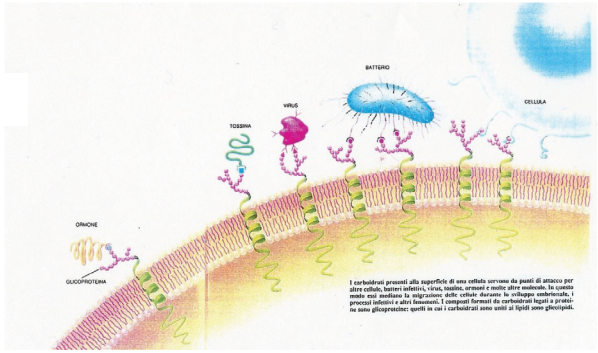


Non Self epitopes
Tumoral Markers

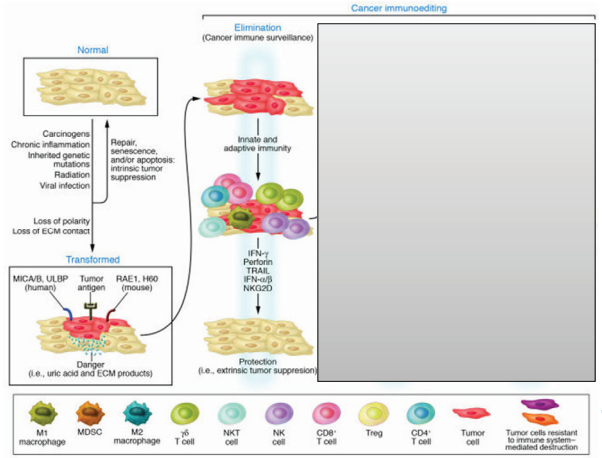


Carbohydrate-shell: the Glycocalix

Molecular Recognition:
Physiological *vs.* Pathological Events



Tumor & Immune System



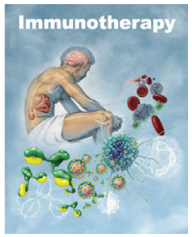
Escape Mechanisms

- Reduce immunogenicity
- Attenuate antitumor Immune response

Is it possible finding a work around?

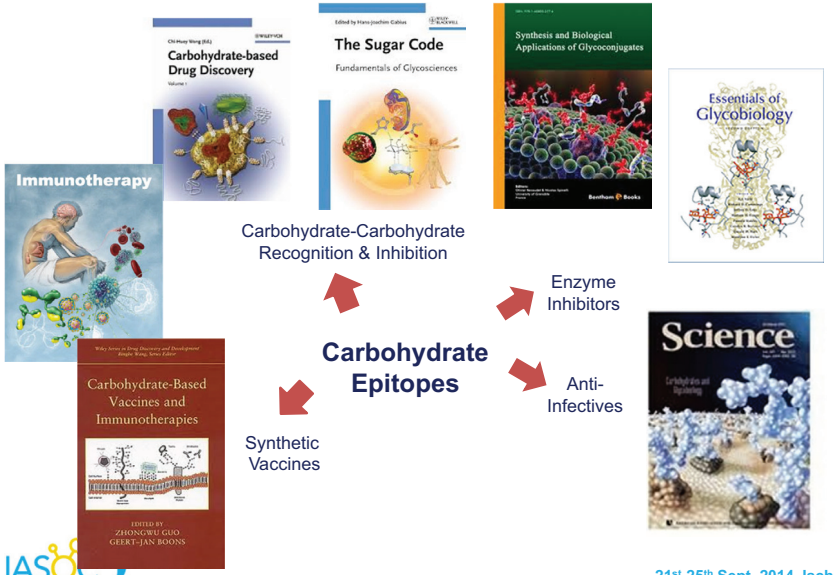
Tumor & Immune System

Chemiotherapy
Radiation theraphy
Biological therapies
Immunotherapy



from *Daily News Global* June 2011

Immunotherapy : Stimulate the tumour-bearing host immune system, making it able to recognize tumour antigens and fight against neo-plastic cells.



Carbohydrate-Carbohydrate Recognition & Inhibition

Enzyme Inhibitors

Carbohydrate Epitopes

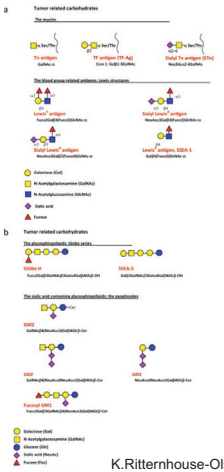
Anti-Infectives

Synthetic Vaccines

Pathogen	Binding epitope	Refs
Bacteria		
Escherichia coli type 1 pili	Manα(1-3)Manα(1-6)Man	109
Helicobacter pylori	Neu5Acα(2-3)Galβ(1-4)Glc	109
Pseudomonas aeruginosa (PA-IL and PA-IIL)	α-Galactoside and Lewis^a	109
Neisseria gonorrhoea	Galβ(1-4)GlcNAc	109
E. coli K99	Neu5Gcα(2-3)Galβ(1-4)Glc	109
E. coli CFA1	Neu5Acα(2-8)Neu5Ac	109
Klebsiella pneumonite	Man	109
Campylobacter jejuni	Fucα(1-2)Galβ(1-4)GlcNAc	109
E. coli K1	GlcNAcβ(1-4)GlcNAc	109
E. coli P	Galα(1-4)Gal	109
E. coli S	Neu5Acα(2-3)Galβ(1-4)GalNAc	109
Haemophylus influenzae	Neu5Acα(2-3)Galβ(1-4)GlcNAc	109
Neisseria meningitidis	Neu5Acα(2-3)Galβ(1-4)GlcNAc	109
Salmonella typhimurium	Man	109
Streptococcus pneumoniae	Neu5Acα(2-3)Gal	109
Streptococcus suis	Galα(1-4)Galα(1-4)Glc	109
Viruses		
Influenza A	α(2-3)- or α(2-6)-linked sialic acid	186
Human parainfluenza virus type 1	α(2-3)SLN and sialyl Lewis^x	187
Norwalk virus	H and Le^b blood types	188
Rotavirus	Sialylated glycans	189
Herpes simplex virus type 1	3-O-sulphated heparin sulphate	190
Calcivirus	Blood group antigens	191
Corona virus	Sialylated glycans	192
Murid herpes virus	Glycosaminoglycans	193
Coxsackivirus A24	Sialylated glycans	194
Papilloma virus L1	Heparan sulphate	195
Polyomaviruses (JCV and BKV)	α(2-3)- or α(2-6)-linked sialic acid	196
Simian virus SV40	GM1 ganglioside	197
Newcastle disease virus	Sialylated glycans	198

BKV, B. K. virus; JCV, John Cunningham virus; Le^b, Lewis^a antigen; PA-IIL, fucose-binding lectin PA-IIL; PA-IL, PA-I galactophilic lectin; SLN, sialyl lactoseamine.

Beat Ernst & John L. Magnani
Nature Reviews Drug Discovery, 8, 661-677, **2009**

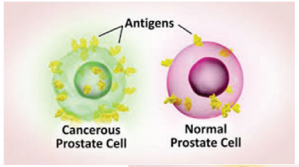


K.Ritternhouse-Olson et al.
Vaccine, 29, 8802-8826, **2011**

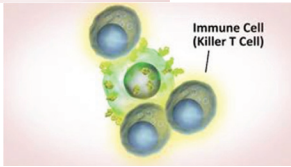
Tumour Associated Carbohydrates Antigens (TACAs)

- ✓ Mucin-related TACAs (Tn, STn & TF)
- ✓ Blood group Lewis-related TACAs (SLeA, SLeX)
- ✓ Globo-series (Globo H)
- ✓ The gangliosides (GD2, GD3, GM1, GM2, GM3 and NeuGcGM3)

Prognostic



Therapeutic Vaccines



Tumor & Immune System

Carbohydrate Epitopes → **Carbohydrate Mimetics**

- ✓ **GM_3 lactone mimetic** (melanoma, colon, lung)
- ✓ **α-Tn mimetic** (breast, colon, pancreas ..)

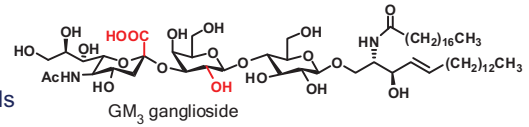
".. The selection of a vaccine target is based not only on the presence of the antigen on a variety of tumour tissue but also on the role this antigen plays in tumour growth and metastasis ..."

Heimburg-Molinaro, J., Ritterhouse-OlsonK. et al. Cancer vaccines and carbohydrate epitopes. *Vaccine*, **2011**, *29*, 8802.

Gangliosides as TAAs

Melanocites *vs.* Melanoma

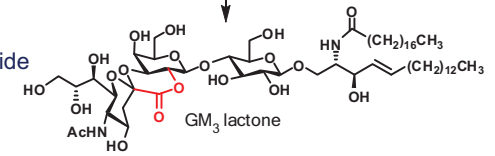
- High density of GM_3
- Lower pH of cancer cells



GM_3 ganglioside

"In vivo" lactonization

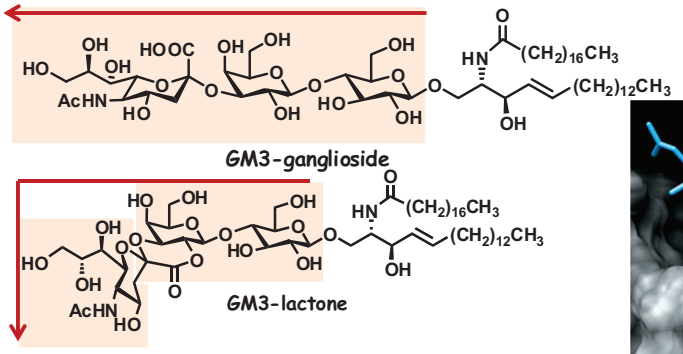
GM_3 lactone is 10 times more immunogenic than GM_3 ganglioside

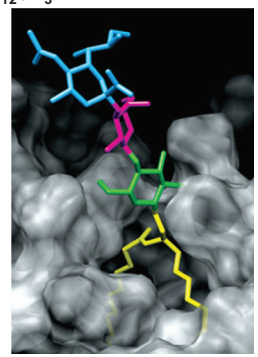


GM_3 lactone

Singhal, A. and Hakomori, S. Molecular changes in carbohydrate antigens associated with cancer. *BioEssay*, **1990**, *12*, 223.

Gangliosides as TAAs

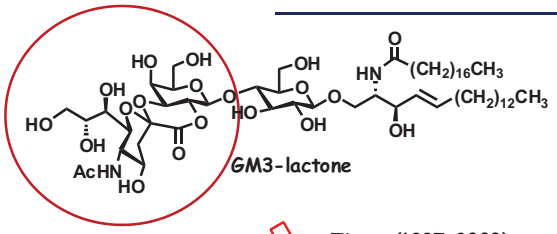




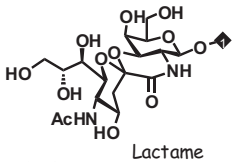
Ganglioside GM3 in a lipid bilayer (gray) from a molecular dynamics simulation using the GLYCAM force field.

DeMarco, M. D., and Woods, R. J. Glycobiol., 2009, 19, 344-355

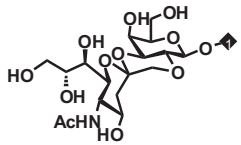
Under physyological pH GM_3-lactone is hydrolitically unstable and unfit for any possible use in immunotherapy



Magnusson et al. (1992, 1995)

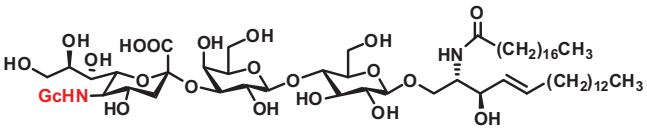


Tietze (1997, 2000)



Gangliosides as TAAs

State of the Art

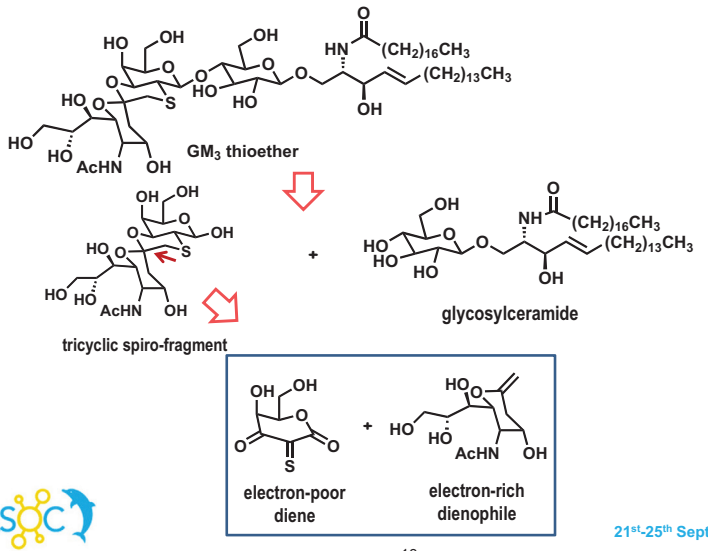


N-glycolyl GM_3 ganglioside

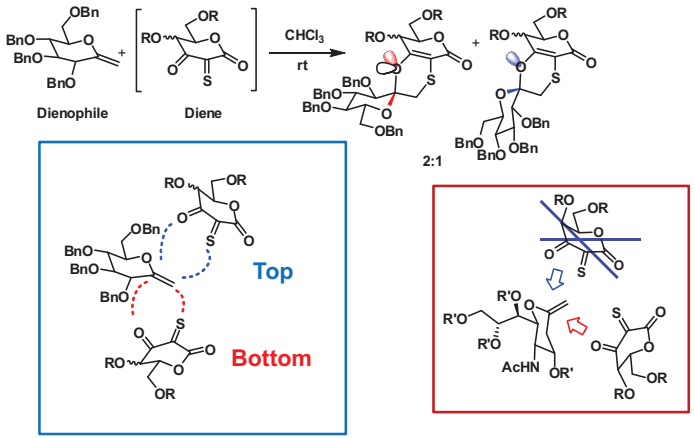
N-glycolyl GM3/VSSP NGcGM3/ very small size proteoliposomes
Approved in Argentina in 2013 for lung cancer

K. Perez, J. Hernandez, L.E. Fernandez et al.
Cancer Menagement and Research **2012**, *4*, 341.345.

A Thioether-bridged GM3 Lactone Mimetic

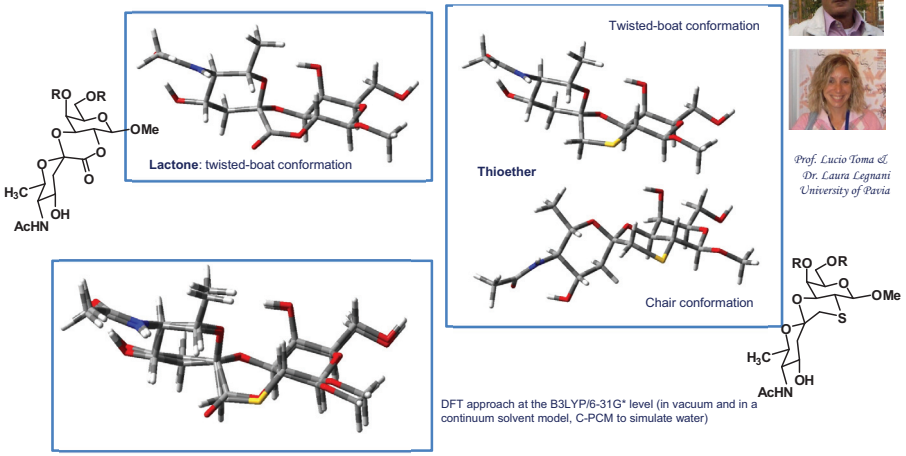


Inverse Electron Demand [4+2] Cycloaddition



G. Capozzi, R.W. Franck , C. Marzabadi, S. Menichetti, C. Nativi et al. *Angew. Chem. Int. Ed.*, **1996**, *108*, 805.
G. Capozzi, S. Menichetti, C. Nativi et al. *Chem. Eur. J.*, **1999**, *5*, 1748.
A. Bartolozzi, S. Menichetti, C. Nativi et al. *J. Org. Chem.*, **2003**, *68*, 8529.

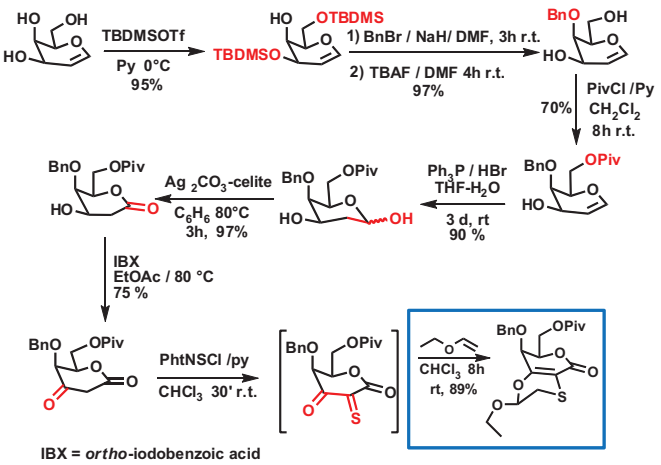
A Thioether-bridged GM3 Lactone Mimetic



Prof. Lucio Toma &
Dr. Laura Legnani
University of Pavia

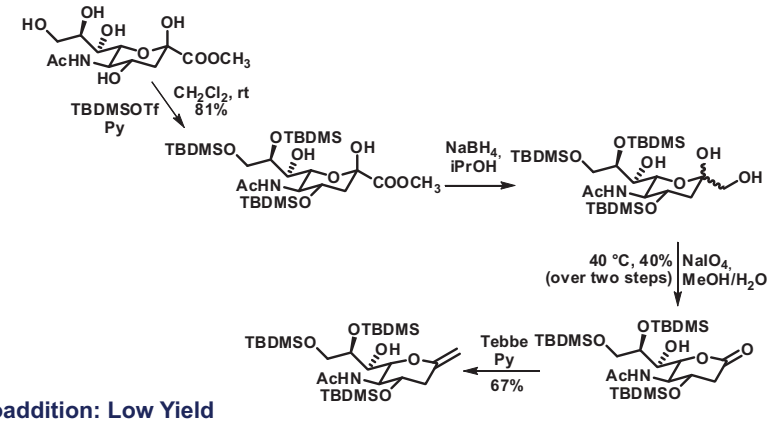
DFT approach at the B3LYP/6-31G* level (in vacuum and in a continuum solvent model, C-PCM to simulate water)

Synthesis of the Electron-poor Diene



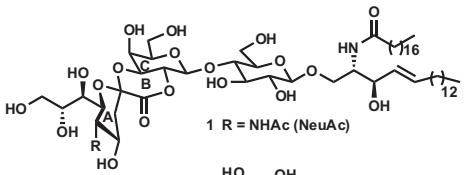
A. Bartolozzi, G. Capozzi S. Menichetti, C. Nativi
Org.Lett., **2000**, *1*, 233.

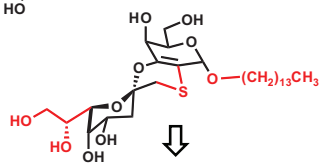
A Thioether-bridged GM3 Lactone Mimetic



Cycloaddition: Low Yield
& Two Unseparable Stereoisomers

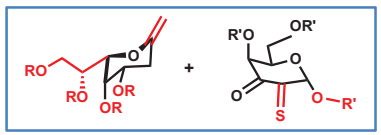
A Thioether-bridged GM3 Lactone Mimetic



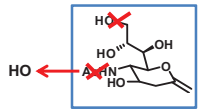


Prof. Mugnai, Dr. Hakomori and
Dr. Kiyotoshi Sekiguchi

Electron-rich
Dienofile

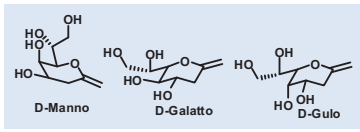


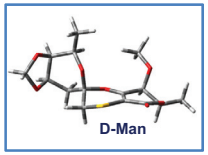
Electron-poor
Diene

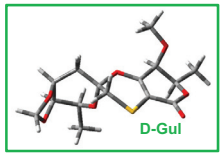


- ✓ Stereochemistry of the stereocentres
- ✓ Top or bottom attach
- ✓ Conformation of the cycloadduct

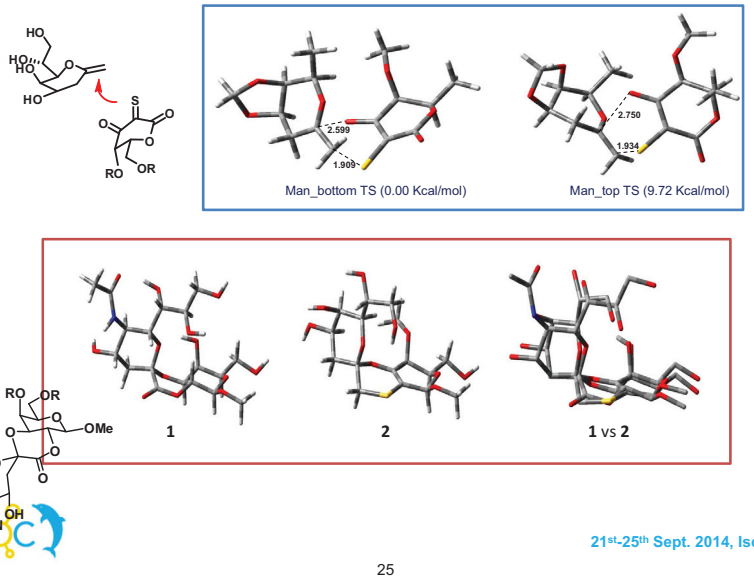
Prof. Lucio Toma & Dr. Laura Legnani
University of Pavia





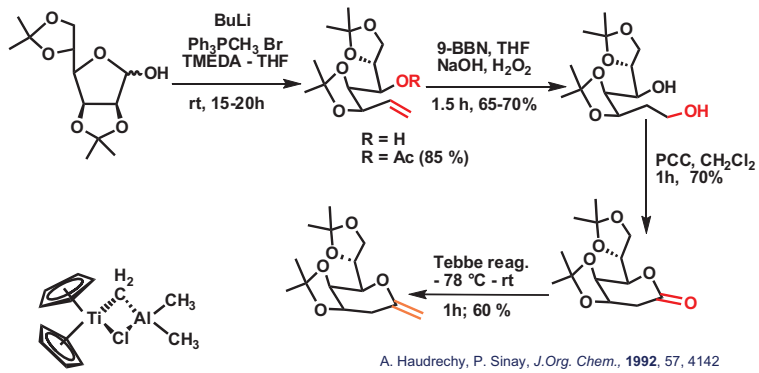


DFT approach at the B3LYP/6-31G* level (in vacuum and in a continuum solvent model, C-PCM to simulate water)



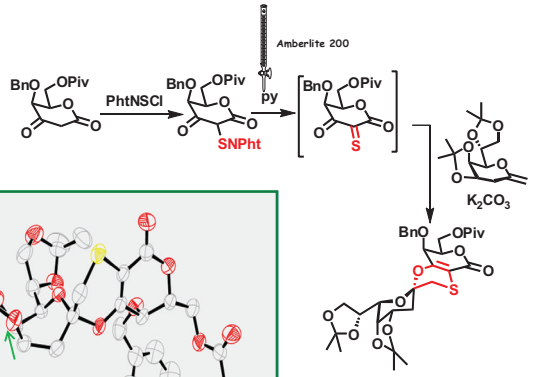
Synthesis of the Electron Rich Heterodiene

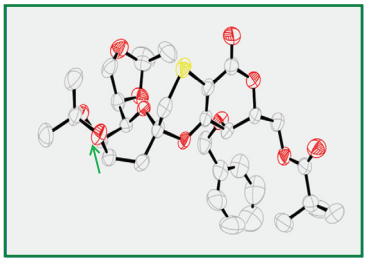
Dr. B. Richichi
University of Florence



A. Haudrechy, P. Sinay, *J.Org. Chem.*, **1992**, 57, 4142

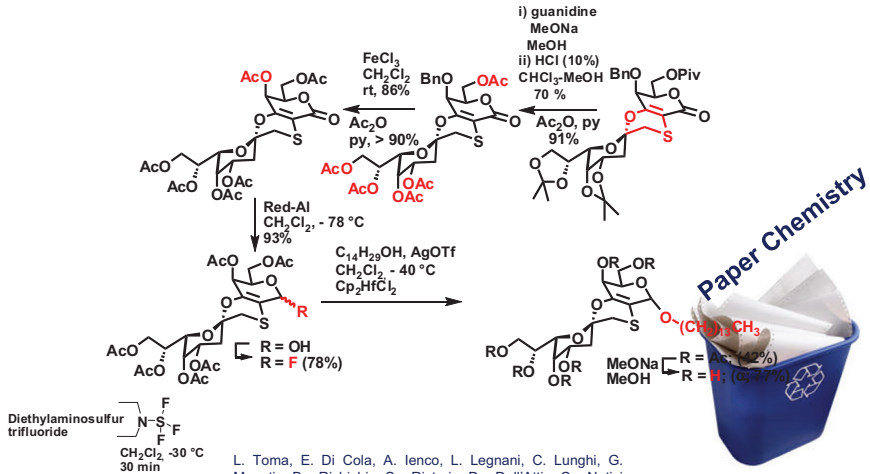
A Thioether-bridged GM3 Lactone Mimetic





Dr. Andrea Ienco
ICCOM (CNR Florence)

A Thioether-bridged GM3 Lactone Mimetic

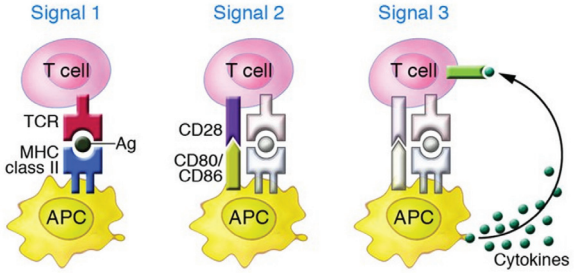


L. Toma, E. Di Cola, A. Ienco, L. Legnani, C. Lunghi, G. Moneti, B. Richichi, S. Ristori, D. Dell'Atti, C. Nativi *ChemBioChem* **2007**, *8*, 1646.

Tumor & Immune System

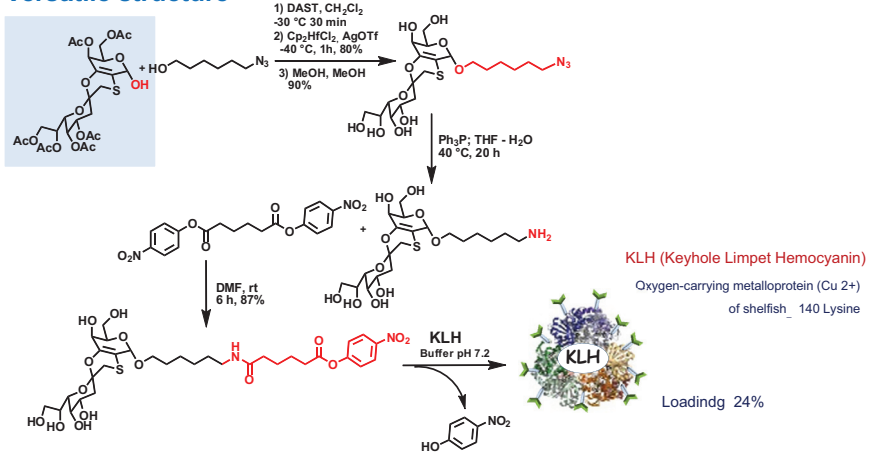
Immune Response is a mutistep process

- ✓ Antigen Recognition (**Signal 1**)
- ✓ Microbes and innate response against them or host dameged cells by pathogens (**Signal 2**) ➡ Pathogen-mediated Signal
- ✓ Limphocytes Activation (adaptive immune response - **Signal 3**)



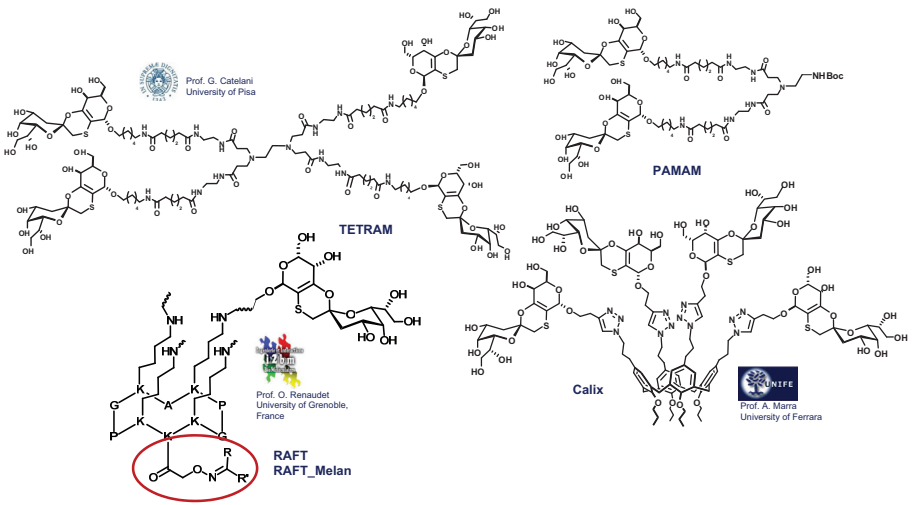
KLH-GM3 Lactone Mimetic Glycoconjugate

Versatile structure

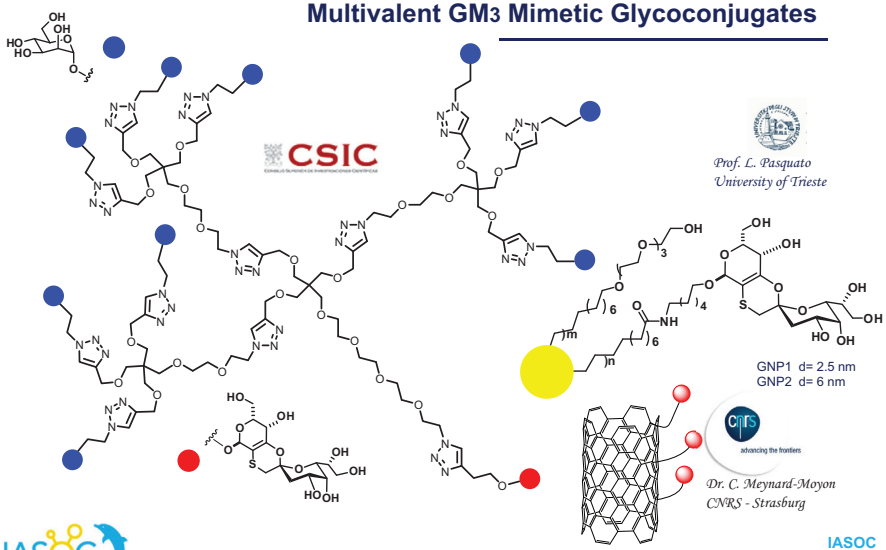


Arcangeli A., Toma L.; Contiero L.; Crociani C.; Legnani L.; Lunghi C.; Nesti E.; Moneti G.; Richichi B.; Nativi C.; *Bioconjugate Chem*, **2010**,*21*, 1432
Avery, O.T.; Geobel, W.F. *J. Exp. Med.* **1929**, *50*, 533.

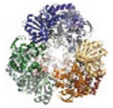
Multivalent GM3 Mimetic Glycoconjugates



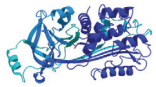
Multivalent GM3 Mimetic Glycoconjugates



GM3 Lactone Mimetic Glycoconjugates

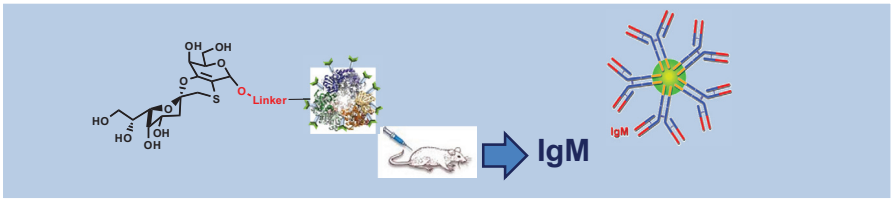


➢ KLH MIM_conjugate



➢ Ova MIM_conjugate

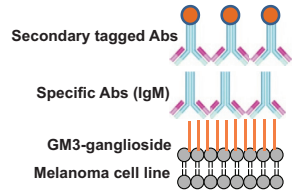
➢ Pam3CysSer MIM_conjugate

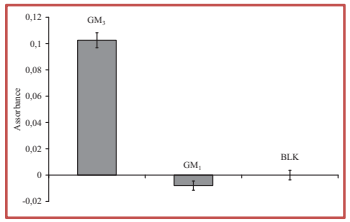


Nativi, C.; Toma, L.; Richichi, B. *PCT n°* 9896 PTWO, **2011.**

GM3 Lactone Mimetic Glycoconjugates

✓ Stable under physiological conditions
✓ MIM_GM-3 antigen raises an immune response "in vivo"
✓ Abs (anti MIM_GM-3) recognize the GM3 iper-expressed on melanoma cells



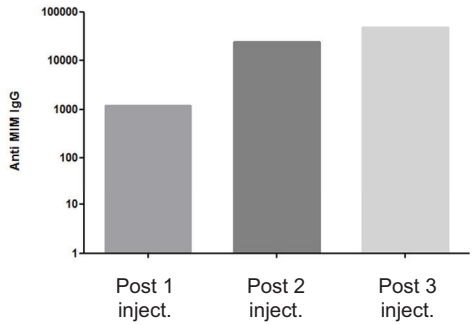


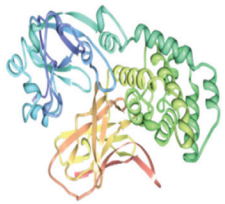
The supernatant binds GM_3 whereas no appreciable binding to GM_1 was observed.

Nativi, C.; Toma, L.; Richichi, B. *PCT n°* 9896 PTWO, **2011.**

CRM197 Glycoconjugates

Coating OVA-MIM 10µg/ml of protein in PBS pH 7.2



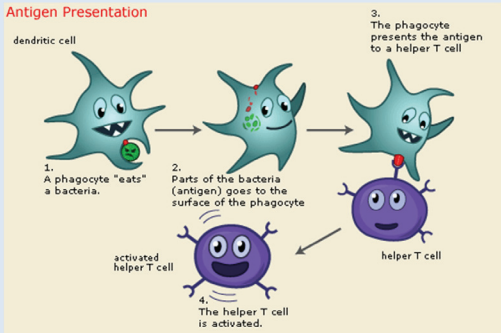


CRM197
Non-toxic mutant of Diphtheria toxin

No aspecific reactions vs. the linker or Ova

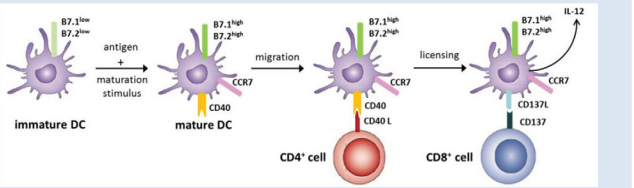
F. Berti, M. Romano, M. Tontini, Novartis Vaccines - Siena

DCs Activation

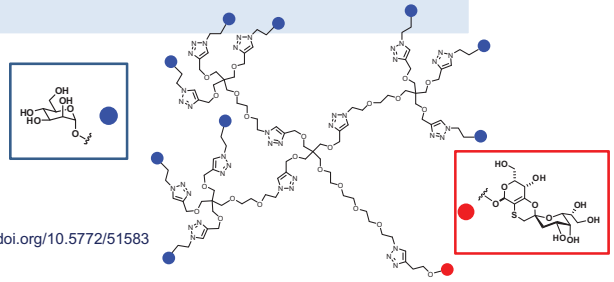


W. De Haes et al. http://dx.doi.org/10.5772/51583

DCs Activation

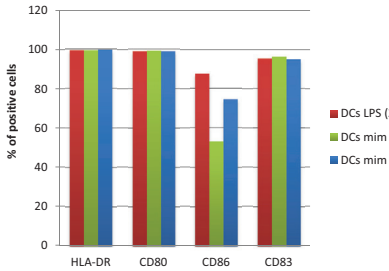


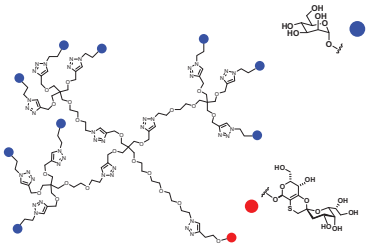
Prof. Javier Rojo
CSIC, Sevilla



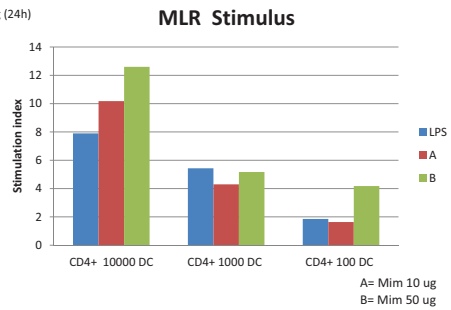
W. De Haes et al. http://dx.doi.org/10.5772/51583

DCs Activation

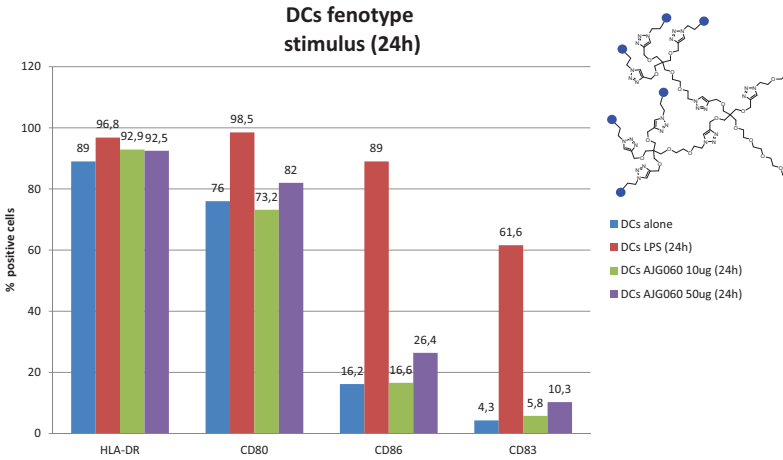


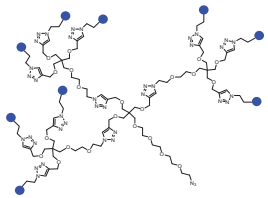


Dr. Clara Ballerini
University of Florence



DCs Activation

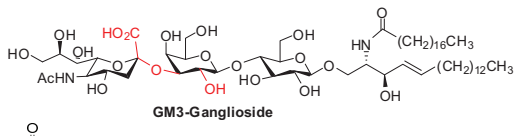




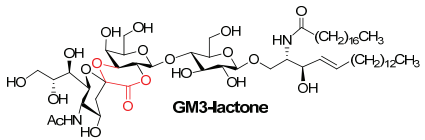
GM3 Ganglioside & Its Biological Functions

.. the **knowledge** of the role that the carbohydrate antigen plays in tumour growth and spread ..
for tumours have the ability to **mutate losing the expression** of a specific antigen **unless** that antigen plays a key role in the tumour spread or adhesion

The molecular mechanism of cancer "malignacy"



GM3-Ganglioside

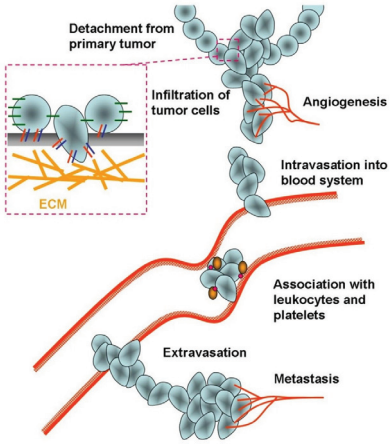


GM3-lactone

The molecular mechanism of cancer "malignacy"

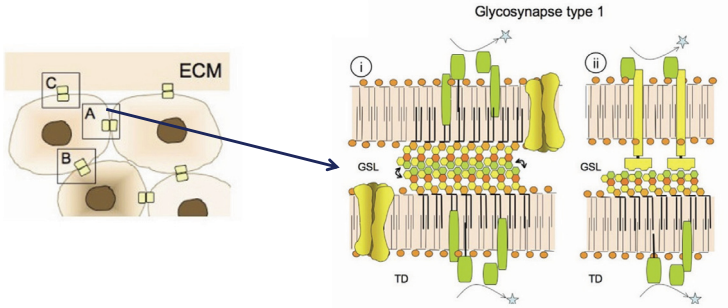
Adhesion / Migration Molecules

- ✓ Integrins
- ✓ Cadherins
- ✓ Galectins
- ✓ Glycosphingolipids (GLS) → GM-3 ganglioside
- ✓ Tetraspanins (TSPs)
- ✓ Grow factors



Micheal, B., Gerd, *Reasearch on melanoma*, **2011** Ed. by Mandi Murph.

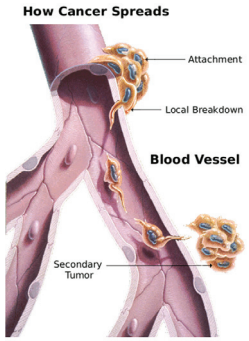
GM3 Ganglioside & Its Biological Functions



GM3 is overexpressed in Glycosynapses where interacts with transmembrane receptors or signal transducers involved in cell adhesion and signaling

S. Hokomori, *PNAS*, **2002**, 99(1), 225-232; S.Hokomori, *FEBS Letters*, **2010**, 584, 1901-1906; A. R. Todeschini, S. Hokomori, *Biochim. Biophys, Acta*, **2008**, 1780(3), 421-433; S. Hokomori, K. Handa, K. Iwabuchi, S. Yamamura, A. Prinetti, *Glycobiology*, **1998**, 8(10), xi-xix.

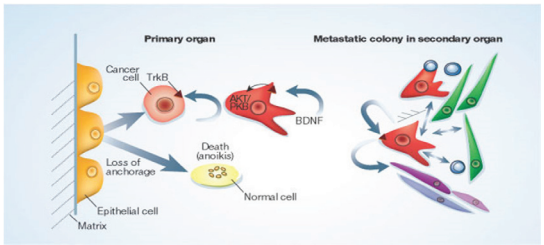
Multivalent GM-3 – containing constructs



→ **Cell Adhesion - Invasion**

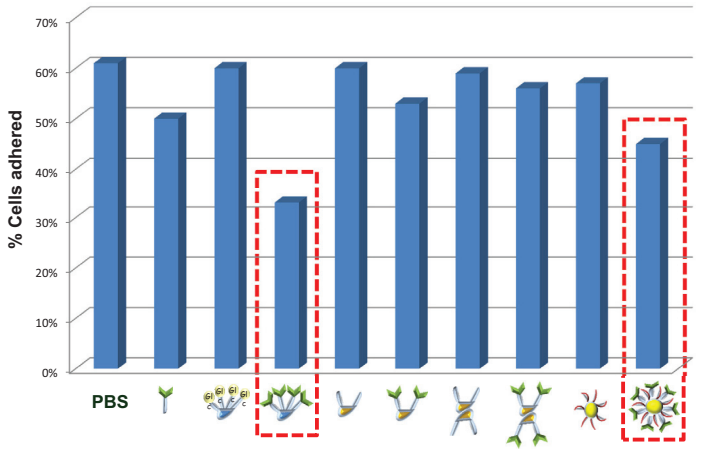
→ **Migration - Metastasis**

Anoikis



Hakomori, S.-I. *FEBS Letters*, **2010**, *584*, 1901.
Rojo, J., Bernad, A., Penadés, S. et al. **2004**, *5*, 291.

Cells Adhesion, Migration & Anoikis

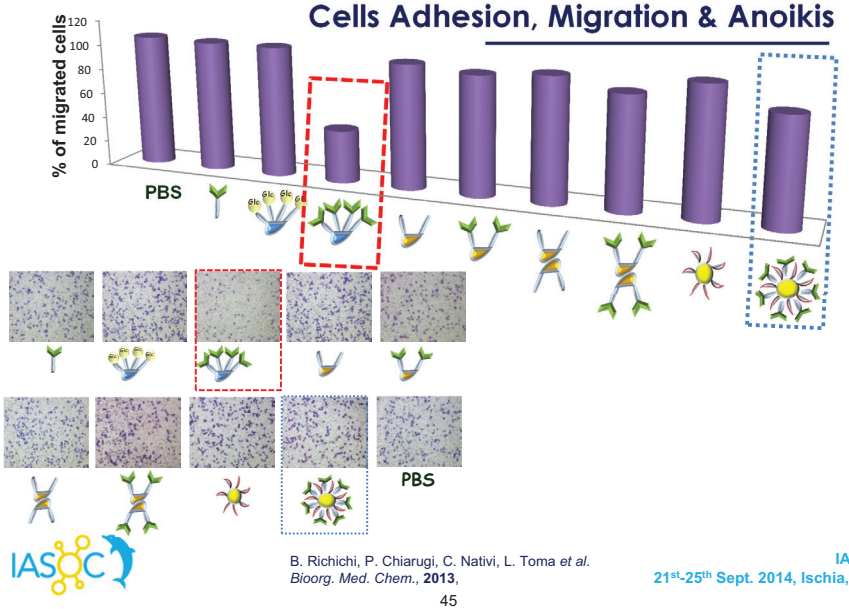


Prof. P. Chiarugi
Dr. G. Comito
University of Florece

A375 cells vs. ECM

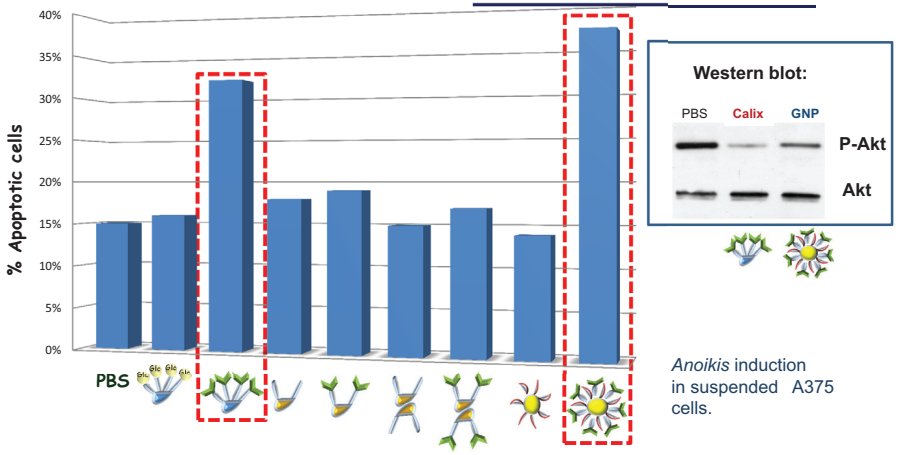
B. Richichi, P. Chiarugi, C. Nativi, L. Toma *et al.* *Bioorg. Med. Chem.*, **2013**,

Cells Adhesion, Migration & Anoikis



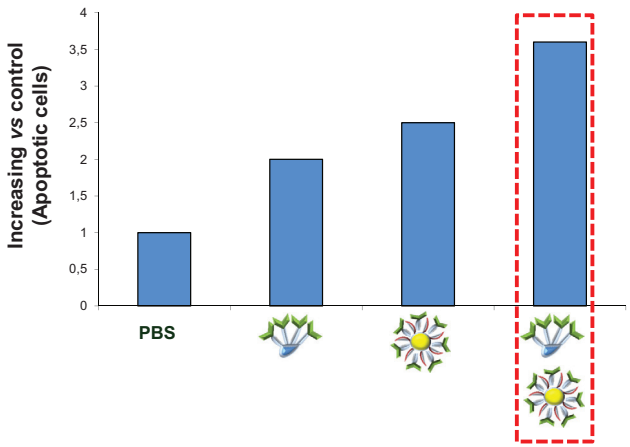
B. Richichi, P. Chiarugi, C. Nativi, L. Toma *et al.* *Bioorg. Med. Chem.*, **2013**,

Cells Adhesion, Migration & Anoikis



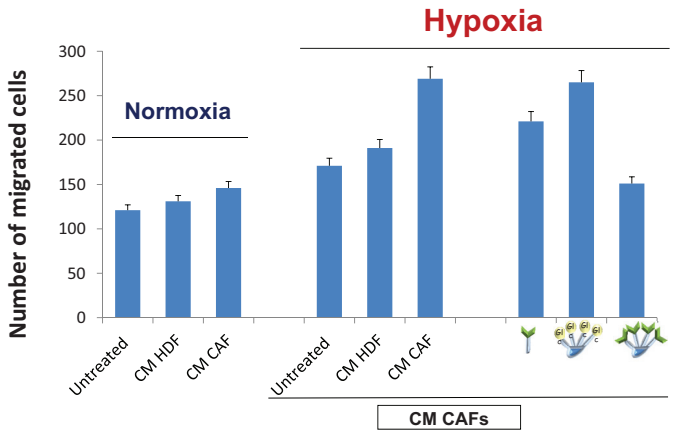
Anoikis induction in suspended A375 cells.

Cells Adhesion, Migration & Anoikis

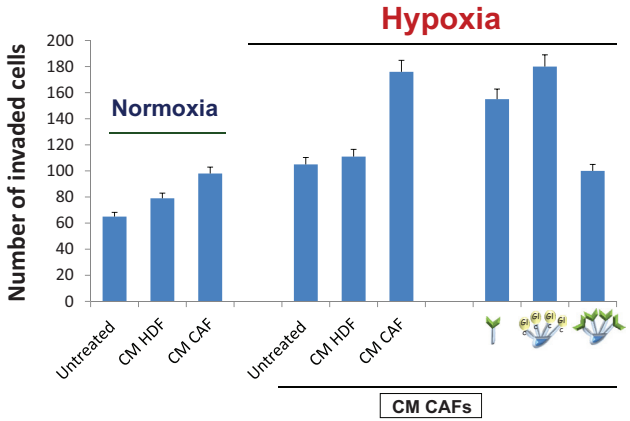


Sinergistic effect Calix & Gold NPs

Migration in Hypoxia



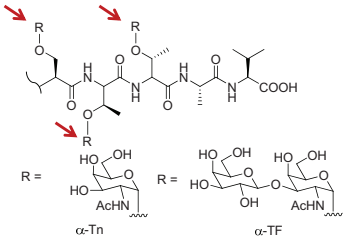
Adhesion in Hypoxia

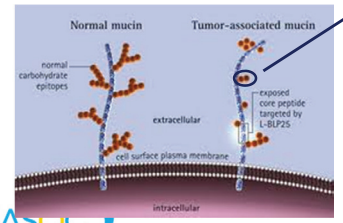


Mucin-antigen type

A large central region formed of multiple tandem repeats of 10 to 80 residue sequences in which up to half of the aminoacids are Ser or Thr

The peptidic backbone is saturated with hundreds of O-linked oligosaccharides





α-TF antigen

Mucin-antigen type

State of the Art

G.J. Boons et al. *PNAS* **2012**, *109*, 261

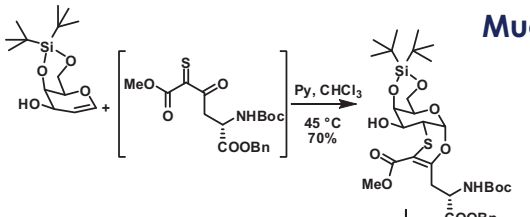
R.J. Payne et al. *Angew Chem Int Ed* **2011**, *50*, 1635

H. Kunz et al. *Angew Chem Int Ed* **2010**, *49*, 3688

A. Zettl, C.R. Bertozzi et al. *Angew Chem Int Ed* **2004**, *43*, 6112.

S.J. Danishefsky et al. *J. Am Chem Soc* **2002**, *124*, 9833

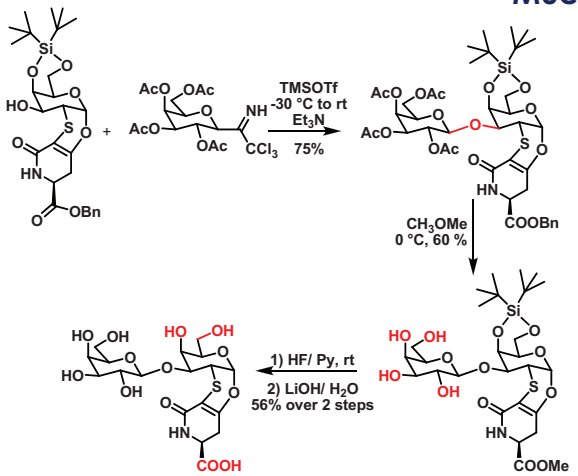
Mucin-antigen type



Dr. Rosa Bosco
University of Florence

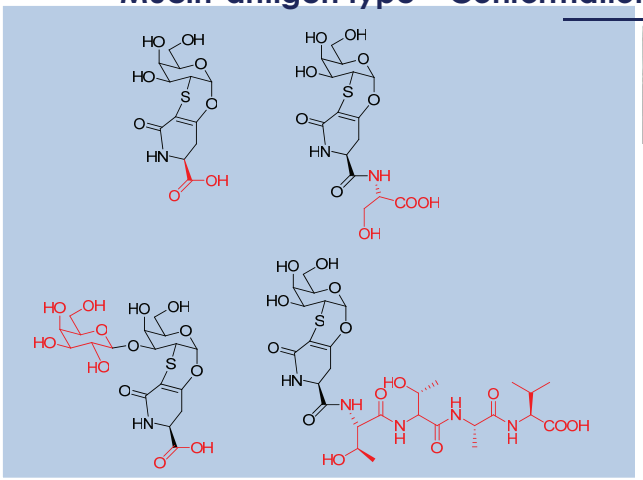
Synthesis of a α-Tn mimetic

Mucin-antigen type



Synthesis of a α-TF mimetic

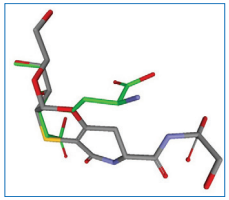
Mucin-antigen type – Conformational Studies



Prof. J.Jimenez Barbero &
Dr. Ana Ardà
CSIC - Madrid

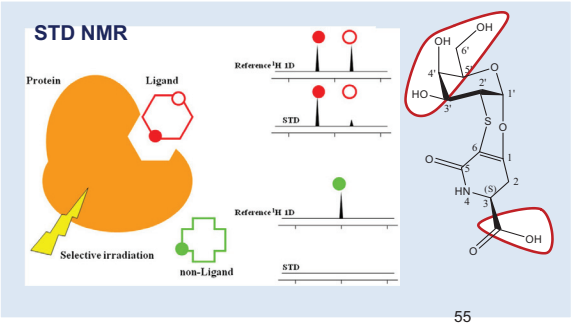
Mucin-antigen type – Conformational Studies

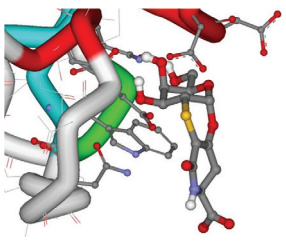
In solution by NMR & Mol. Modeling:
Vicinal proton-proton coupling constants for the pyranose ring of **Gal** residue: unique presence of the $^4C_1$ chair conformation, as expected for natural galactosides.



Interaction with Viscumin Album Agglutinin (VAA)

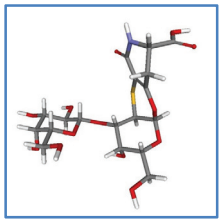
Mim_Tn competes with lactose for the lectin site,
It is recognized as lectin ligand

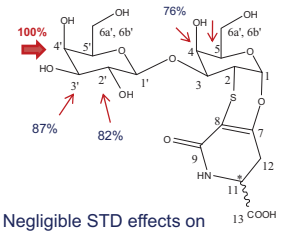




Mucin-antigen type – Conformational Studies

Interaction with Viscumin Album Agglutinin (VAA)





Negligible STD effects on the protons of lactam units

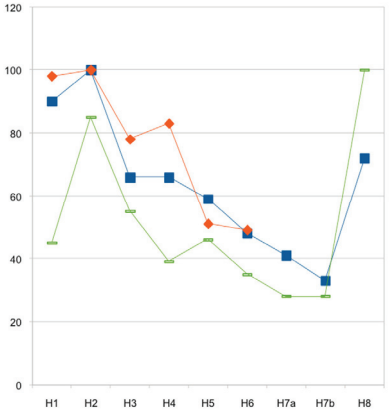
Three Gal specific Lectins

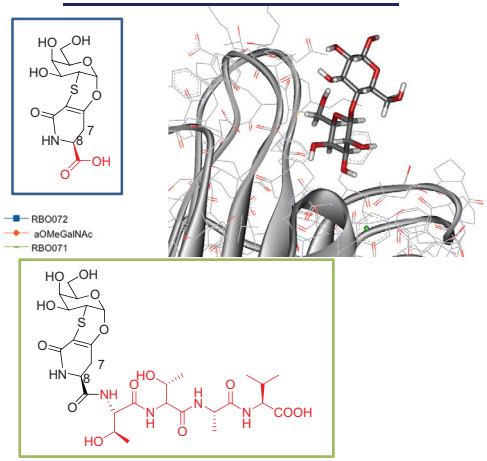
- **Human Macrophage lectin 2 (HML2)** → Gal, GalNAc, Tn
- **Erythrina Cristagalli lectin (ECL)** → Galβ1-4GlcNAc, Galβ
- **Helix Pomatia agglutinin (HPA)** → GalNAcα1-3Gal; β-GalNAc; GlcNAcβ1-4Gal

HML2 lectin provided by Prof. H.J. Gabius as extracellular fragment of HML2 (aa68-316), containing the carbohydrate recognition domain (CRD, aa187-316).

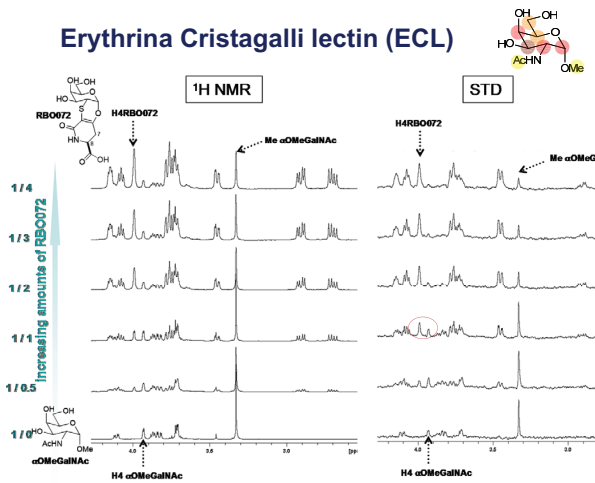
Erythrina Cristagalli Lectin (ECL)

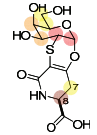
STD Intensity (1:100-ECL:Mim ratio)





Erythrina Cristagalli lectin (ECL)

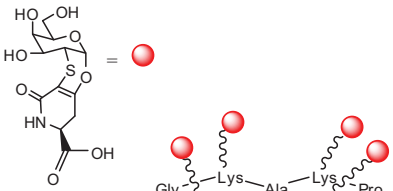


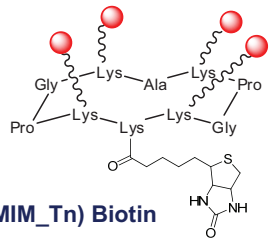


80-100%
80-70%
70-60%
60-40%

- STD competition exps s suggest that the binding site for Tn_Mim and Me Gal is the same
- **Tn_Mim binds better than αOMeGalNAc**

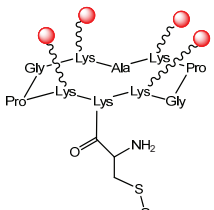
RAFT-Based Multivalent MIM_Tn

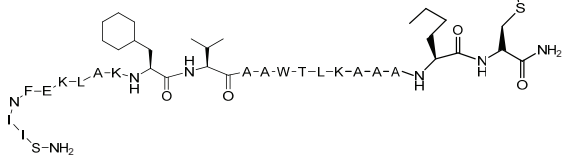




RAFT (4 MIM_Tn) Biotin

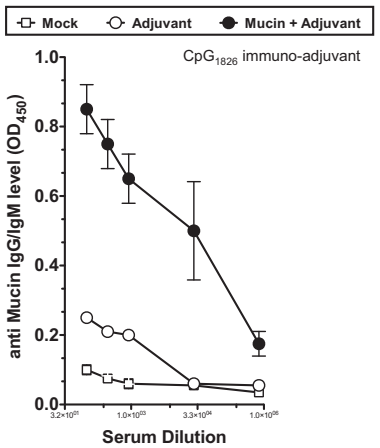
Prof. O. Renaudet
J. Fourier, Grenoble





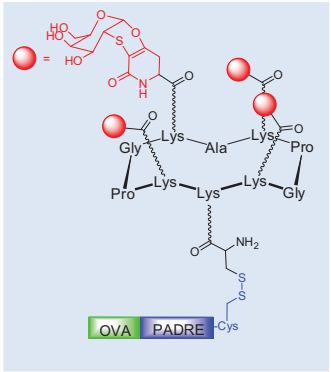
RAFT (4 MIM_Tn) OvaPADRE

RAFT-Based Multivalent MIM_Tn



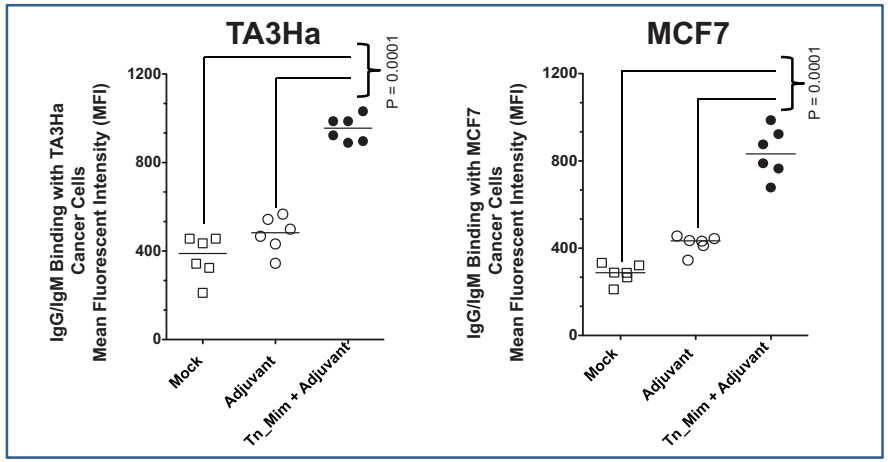
Three groups B6 mice (*n* =10) immunized subcutaneously two times with an interval of 14 days

Dr. Benmohamed Lbashir
Medical School, Irvine



B. Richichi, O. Renaudet, L. BenMohamed, C. Nativi et al. *Angew. Chem Int. Ed.* **2014** in press

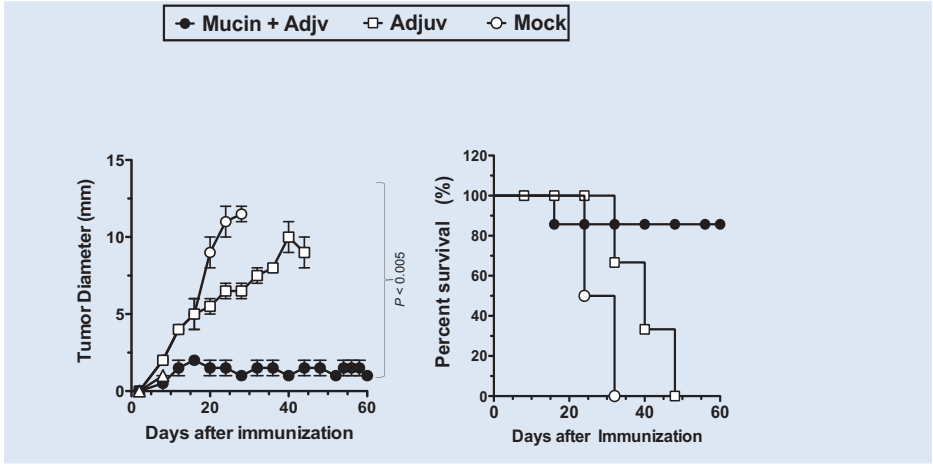
RAFT-Based Multivalent MIM_Tn



TA3Ha mice mammary tuomor cells
MCF7 human mammary tumor cells

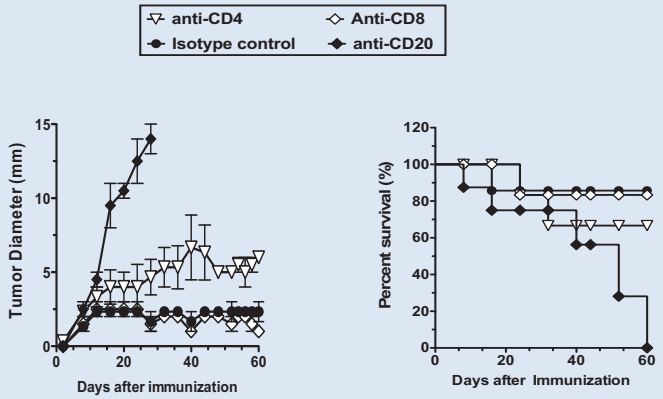
B. Richichi, O. Renaudet, L. BenMohamed, C. Nativi et al. *Angew. Chem Int. Ed.* **2014** in press

RAFT-Based Multivalent MIM_Tn



B. Richichi, O. Renaudet, L. BenMohamed, C. Nativi et al. *Angew. Chem Int. Ed.* **2014** in press

RAFT-Based Multivalent MIM_Tn



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