Immunological Adjuvants
The Indispensable Third Component

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Australian Cancer Inc. (GPI-0100)

Ways to Augment Vaccine Immunogenicity

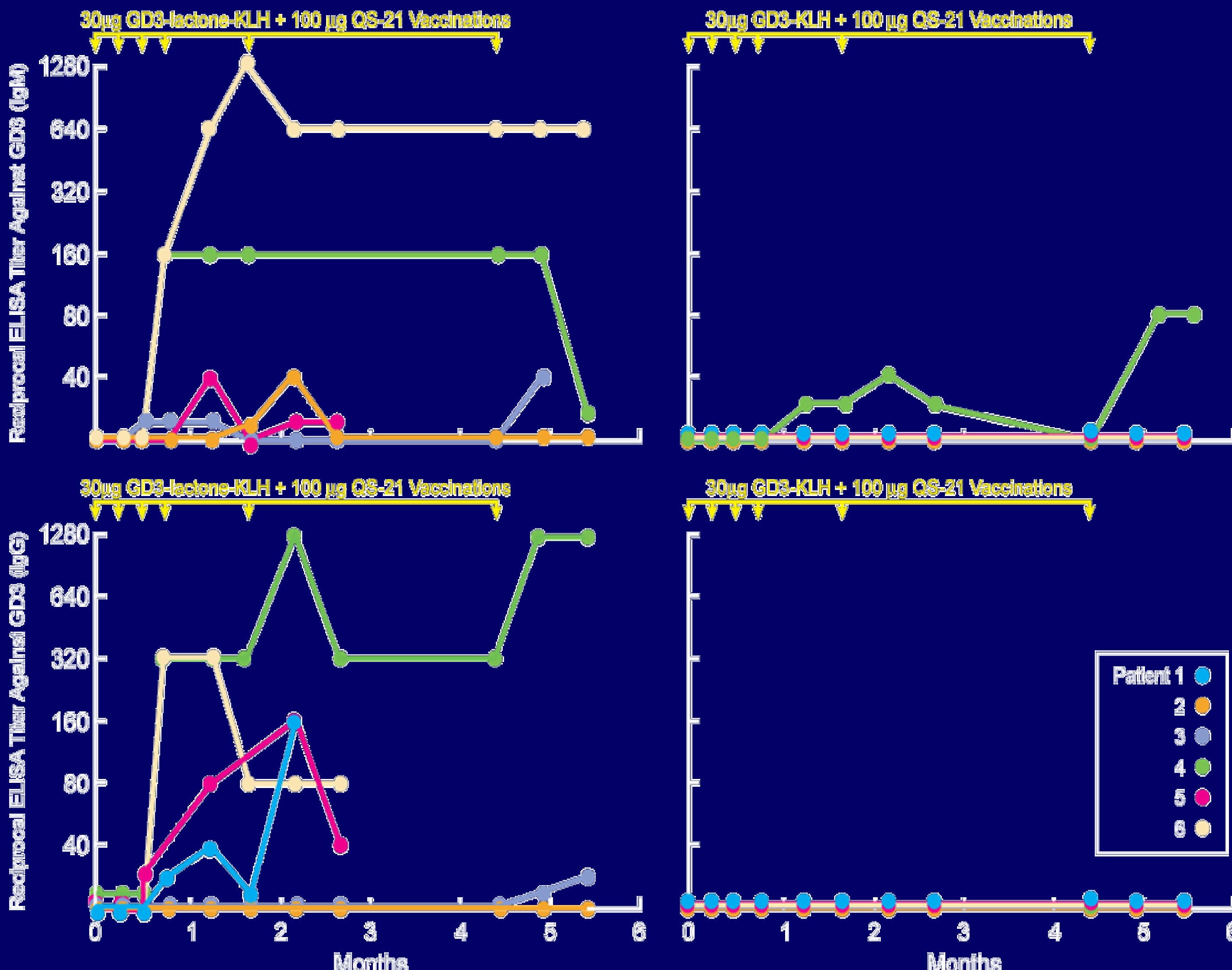
- **Modify the antigenic epitope**

  T-cells: change MHC anchor residues

  B-cells: increase antigen stability – GD2 and GD3 lactones

  xenogenize antigen – N- propionylated polysialic acid

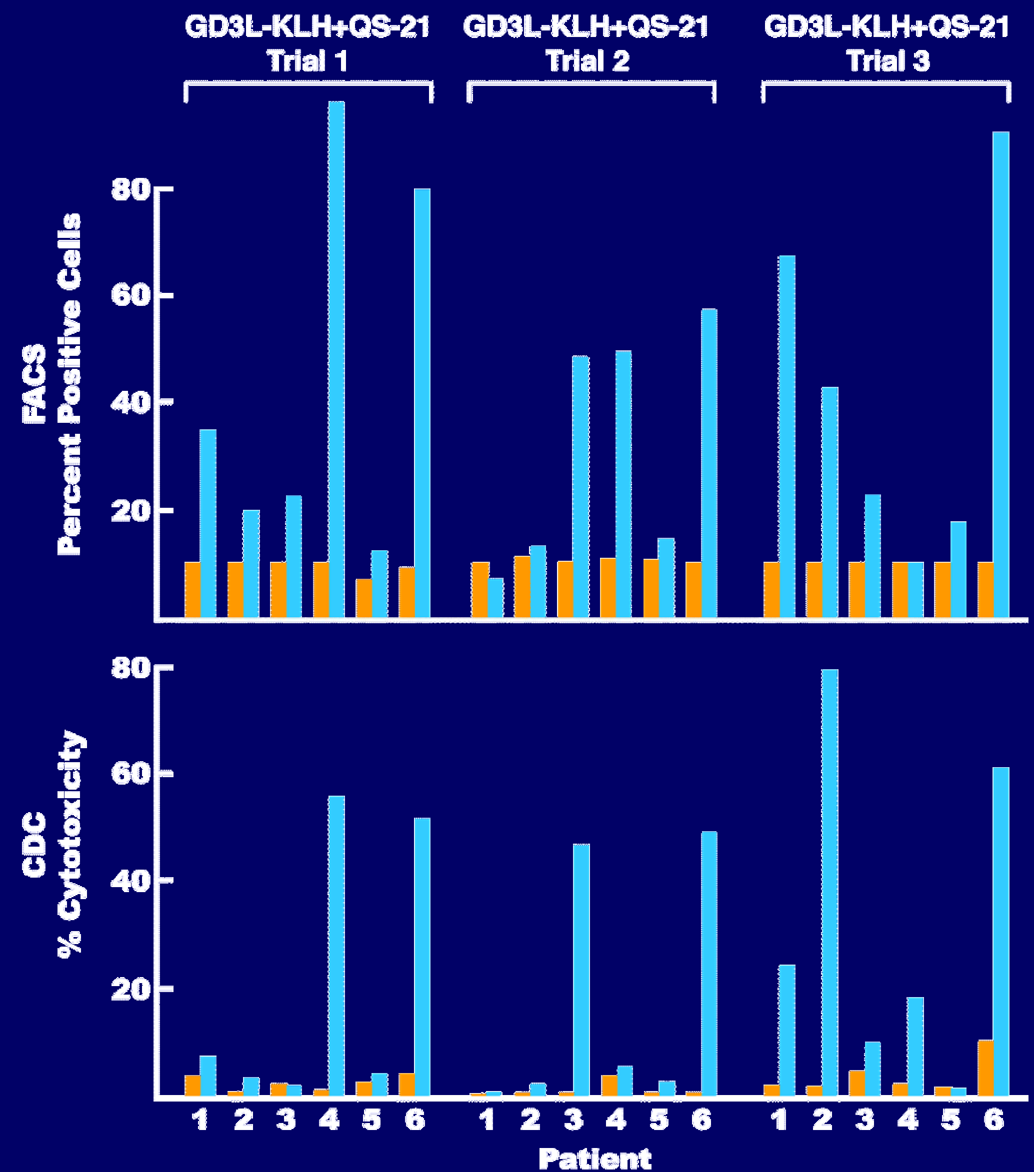
- **Modify nearby components**
- **Immunological adjuvants**
- **Immune modulators**



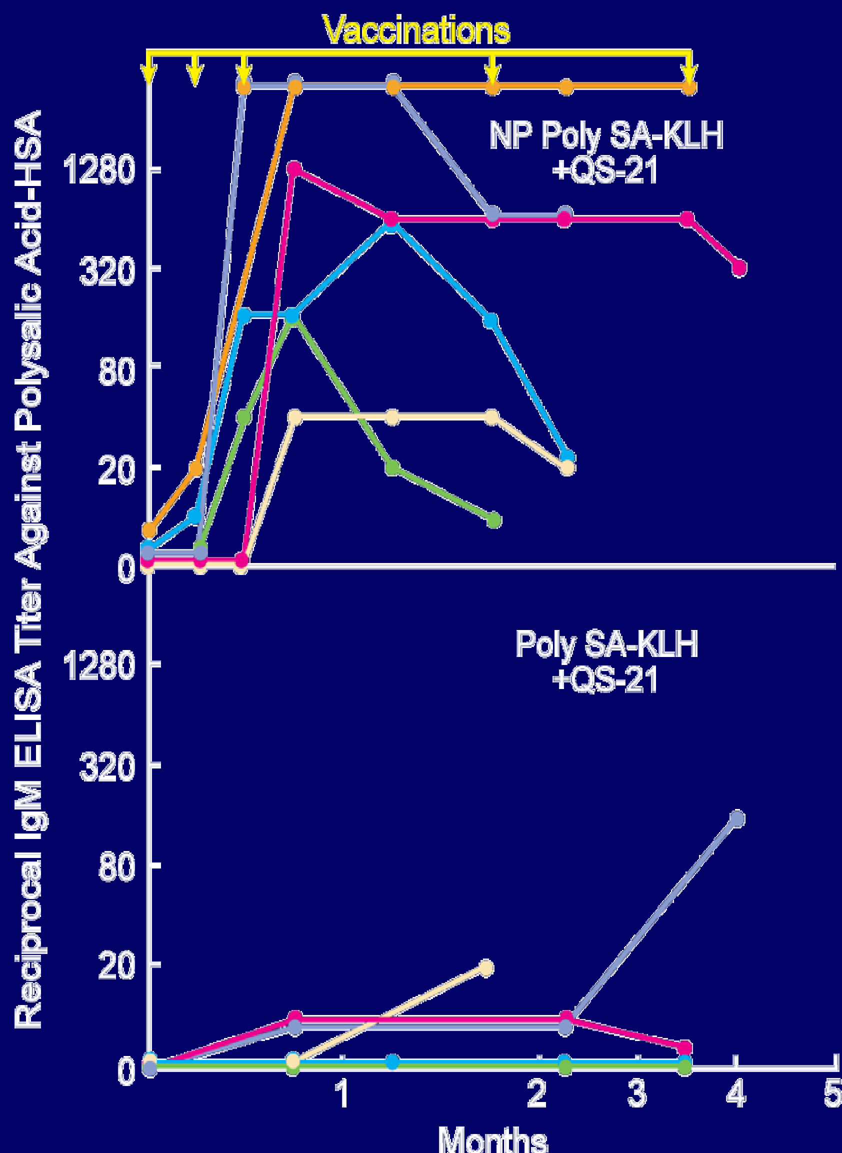
Reactivity against Melanoma Cells

	IgM	IgG	IgM	IgG
ITLC	++	+	–	–
FACS	+		–	–

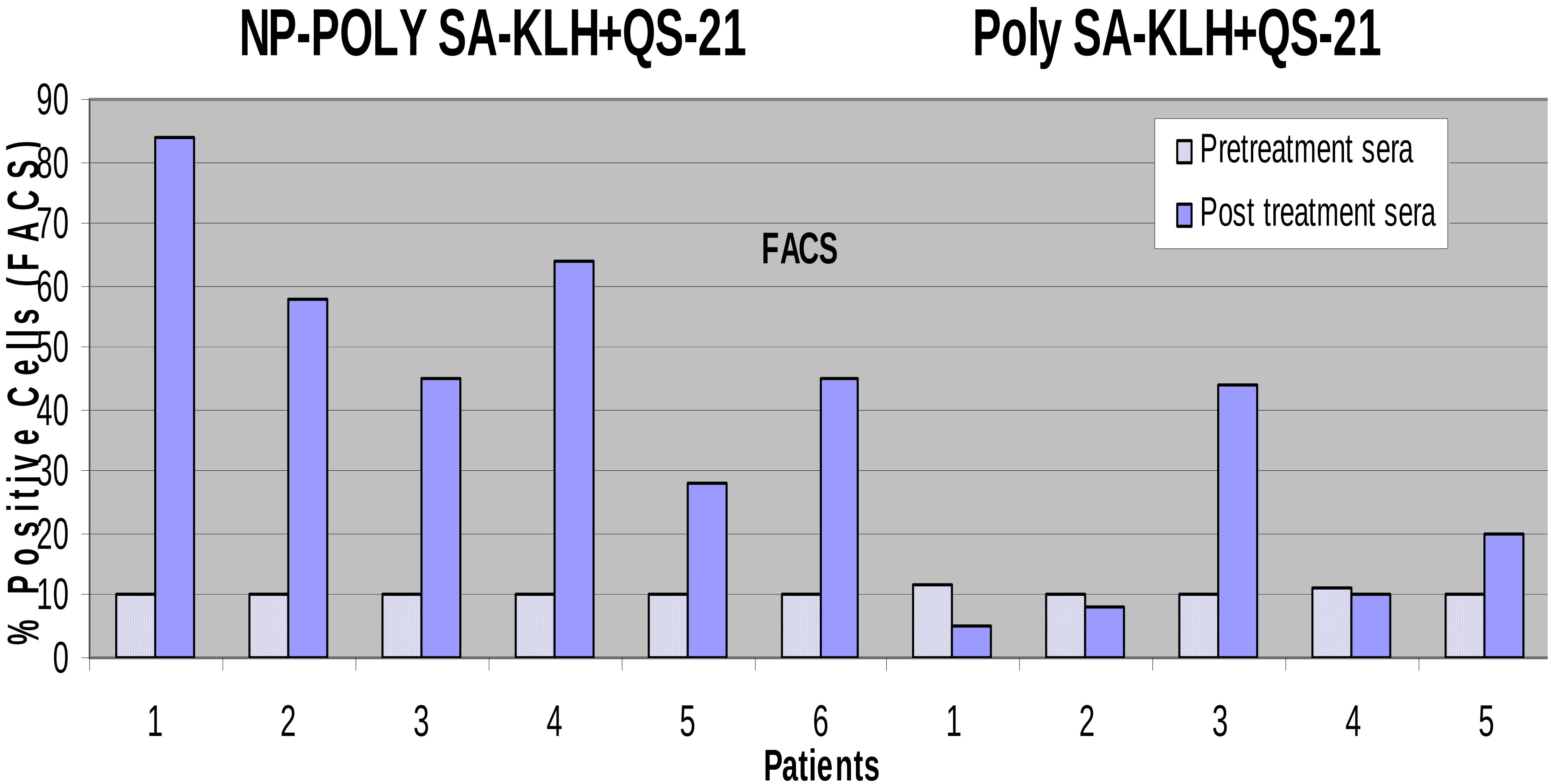
Reactivity Against SK MEL 28 Cells of Sera from Melanoma Patients Vaccinated with GD3L-KLH+QS-21



IgM ELISA Titers Against Polysialic acid in SCLC Patients After Vaccination



Reactivity Against H345 SCLC with Sera from SCLC Patients Vaccinated against Poly Sialic Acid (PolySA)



Ways to Augment Vaccine Immunogenicity

- Modify the antigenic epitope
- Modify nearby components
- Immunological adjuvants
- Immune modulators

Tumor Antigens are Poor Immunogens

1. Because they are autoantigens

   and

2. Because they are surrounded by autoantigens

SOLUTION: XENOGENIZATION

Ways to Augment Vaccine Immunogenicity

- Modify the antigenic epitope

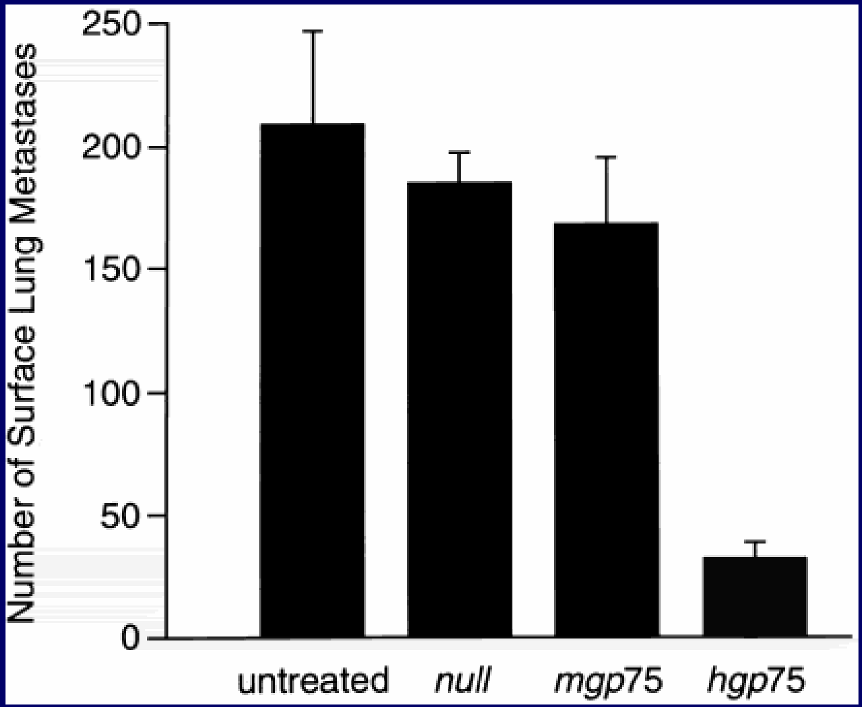
- Modify nearby components

  Xenogenize: T-cell help (conjugate, viral or bacterial vectors)

  Processing: leader sequence, truncation, glycosylation

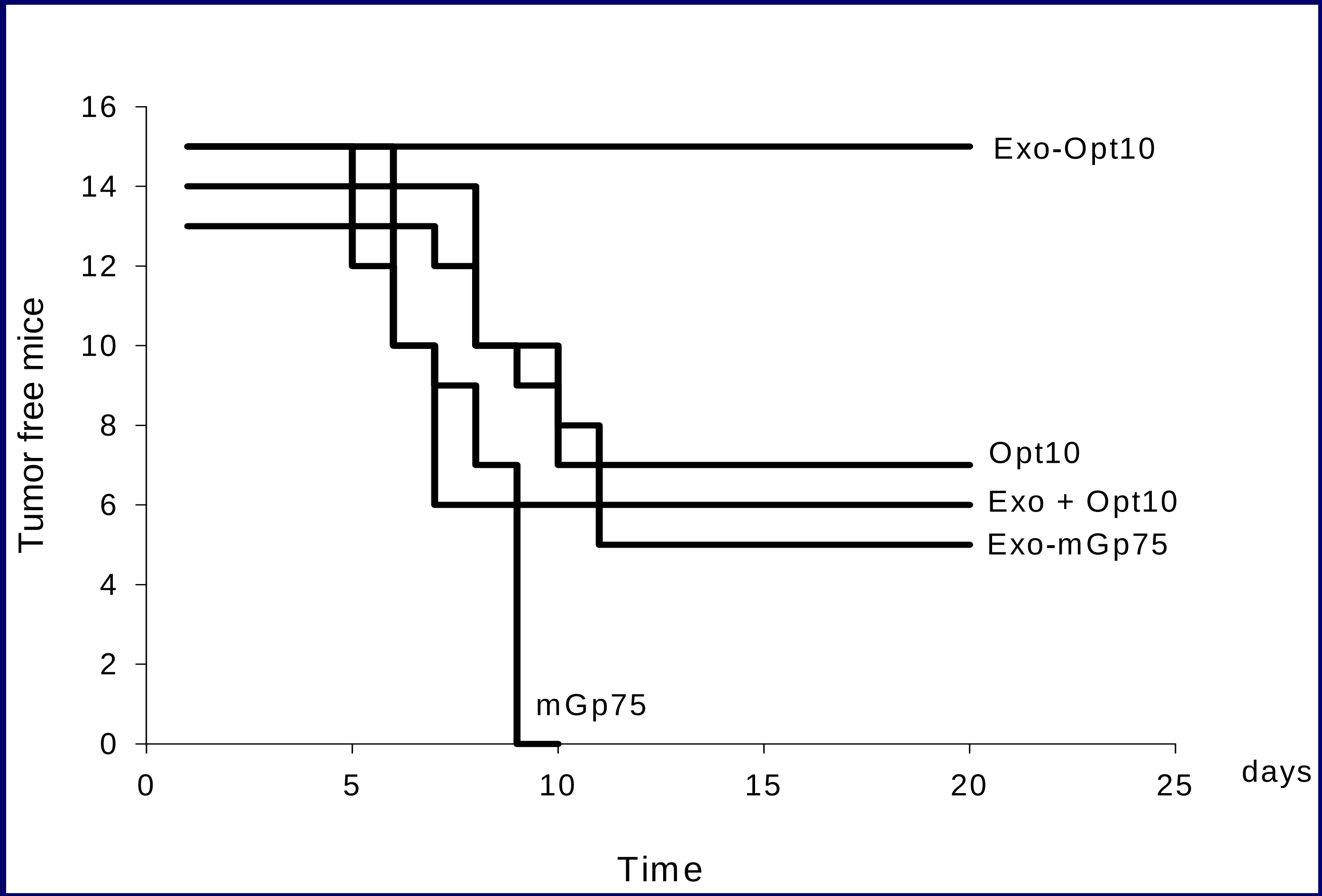
- Immunological adjuvants
- Immune modulators

Tumor protection by genetic immunization with *hgp75*



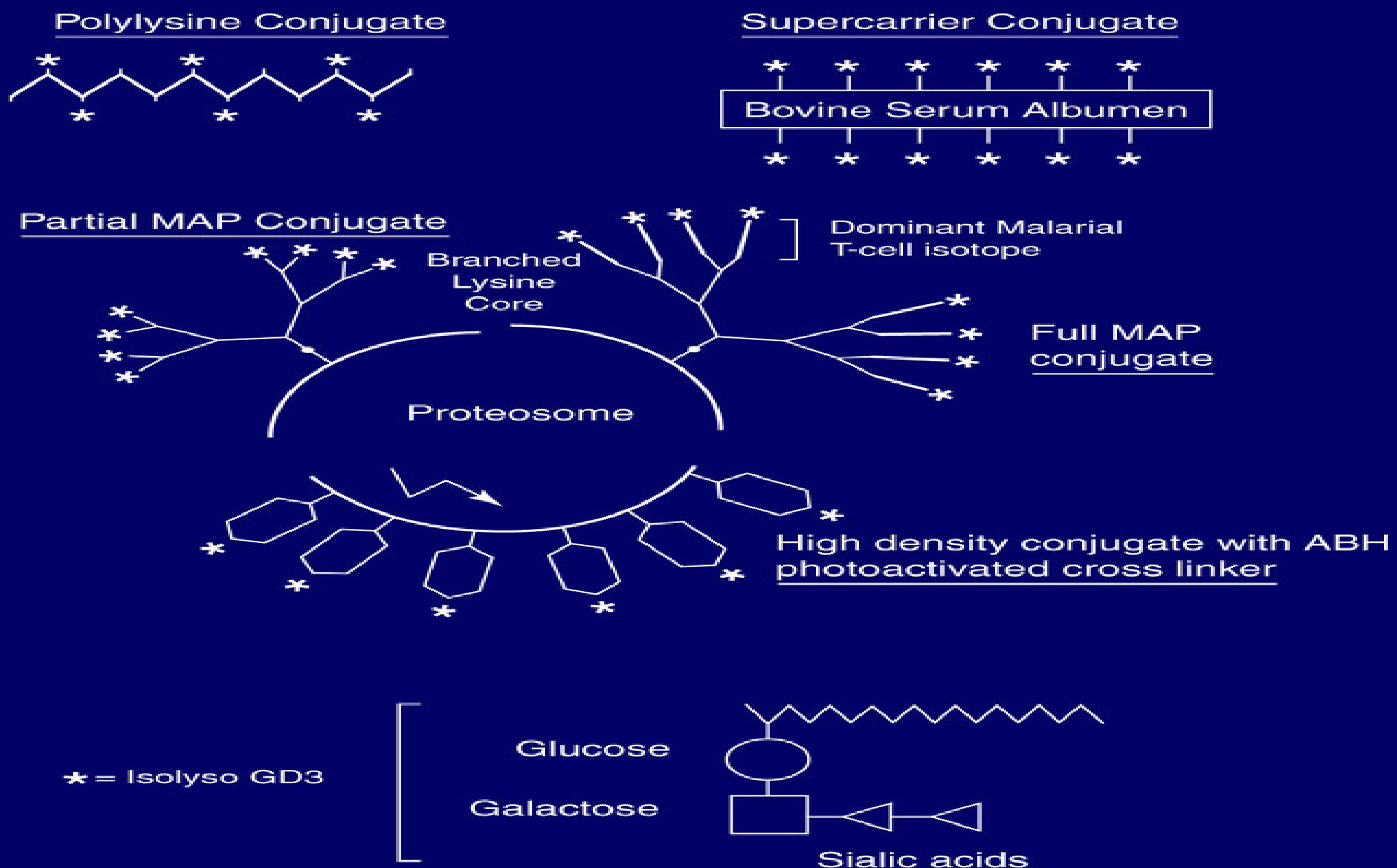
Weber, L. et al. 1998. J.C.I. 102: 1258-1264.

Optimized mGp75 for MHC-I



Guevara, J., Houghton, A.N., et al.

Figure 3
Conjugate GD3 isolysoganglioside vaccines



Conjugate Vaccine Components for Optimal Antibody Induction

Antigen – Carrier (KLH) + Adjuvant (QS-21)

Antigen must mimic expression on tumor cell	Cytokine release is proportional to carrier immunogenicity	Activation of APCs, B-cells and T-cells optimal
High antigen/carrier ratio needed	Cytokine release sequence may be key	Depot effect

Ways to Augment Vaccine Immunogenicity

- Modify the antigenic epitope
- Modify nearby components
- Immunological adjuvants
  - Activate APCs, B and T-cells
  - Depot effect
  - Access to endogenous pathway
- Immune modulators

Requirement for Immunological Adjuvants

Role	Vaccine Type
Definite:	Carbohydrates, Peptides, Proteins Conjugates
	Tumor Cells (BCG)
	DNA (cytokines, electroporation)
None:	Live Vectors DCs

Toll Like Receptors activate NF-κB, Linking Innate and Adaptive Immunity

TLR	Pathogen Associated Molecular Patterns	Pathogen
TLR 2	Bacterial lipoproteins, viral porins, hemagglutinin	Yeast, bacteria, viruses
TLR 3	dsRNA	Viruses
TLR 4	LPS	Bacteria
TLR 5	Bacterial flagellin	Bacteria
TLR 6	LPS	Bacteria
TLR 7	U-rich ssRNA, Imiquimod	Viruses
TLR 8	U-rich ssRNA, Imiquimod	Viruses
TLR 9	CpG DNA	Viruses, bacteria
TLR 11	?	Uropathic bacteria

Immunological Adjuvants

Adjuvants	Cytokine Induction	Depot Effect	Antibody Induction
Aluminum Salts, Emulsions (MF59, Montanide), Liposomes, particles	+	++	+
Saponins (QS-21, GPI-0100)	+++	+	++
Lipid A (MPLA, Detox) (TLR4)	+++	-	++
CpG ODN (TLR9)	+++	-	++
Block Copolymers (Titermax)	++	-	++
Cytokines (GM-CSF, IL2, IL12, IL18)	++	-	++
Imiquimod (Aldara) (TLR7,8)	++	-	+

IFN-γ Secretion Induced by 96 hr *in vitro* Stimulation with KLH After Vaccination of Mice with MUC1-KLH and GD3-KLH Plus Various Adjuvants

Antigen	*InVitro* stimulation KLH
GPI-0100	14,560
CpG ODN	8902
QS-21	6573
MPL-SE	5233
MoGM-CSF	1164
TiterMax-G	694
Saline	180

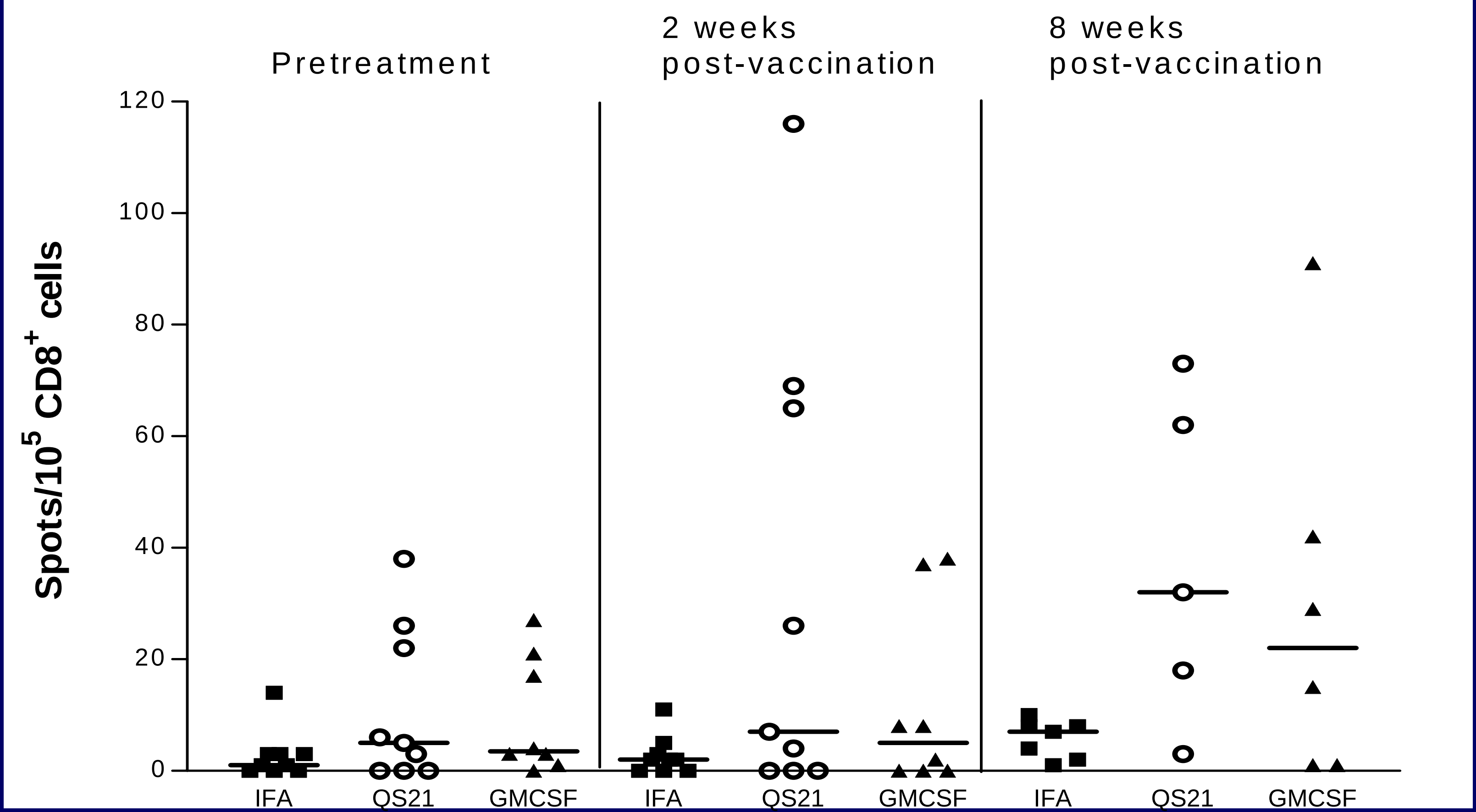
Median Antibody Titer after Immunization of Mice with MUC1-KLH and GD3-KLH Plus Adjuvants

	MUC 1		GD3		
Adjuvant	IgG	IgM	IgG	IgM	Adjuvant
GPI-0100	3277000	12800	450	12150	GPI-0100
QS-21	819200	3200	150	4050	QS-21
Detox-PC	819200	3200	0	4050	TiterMax-G
MPL-SE	819200	3200	0	1350	MoGM-CSF
MoGM-CSF	819200	800	0	1350	Detox-PC
TiterMax-G	204800	3200	0	1350	CpG ODN
CpG ODN	204800	800	0	930	PG-026
Saline	100	400	0	0	Saline

Median Antibody Titer after Immunization of Patients with GM2-KLH plus Different Adjuvants

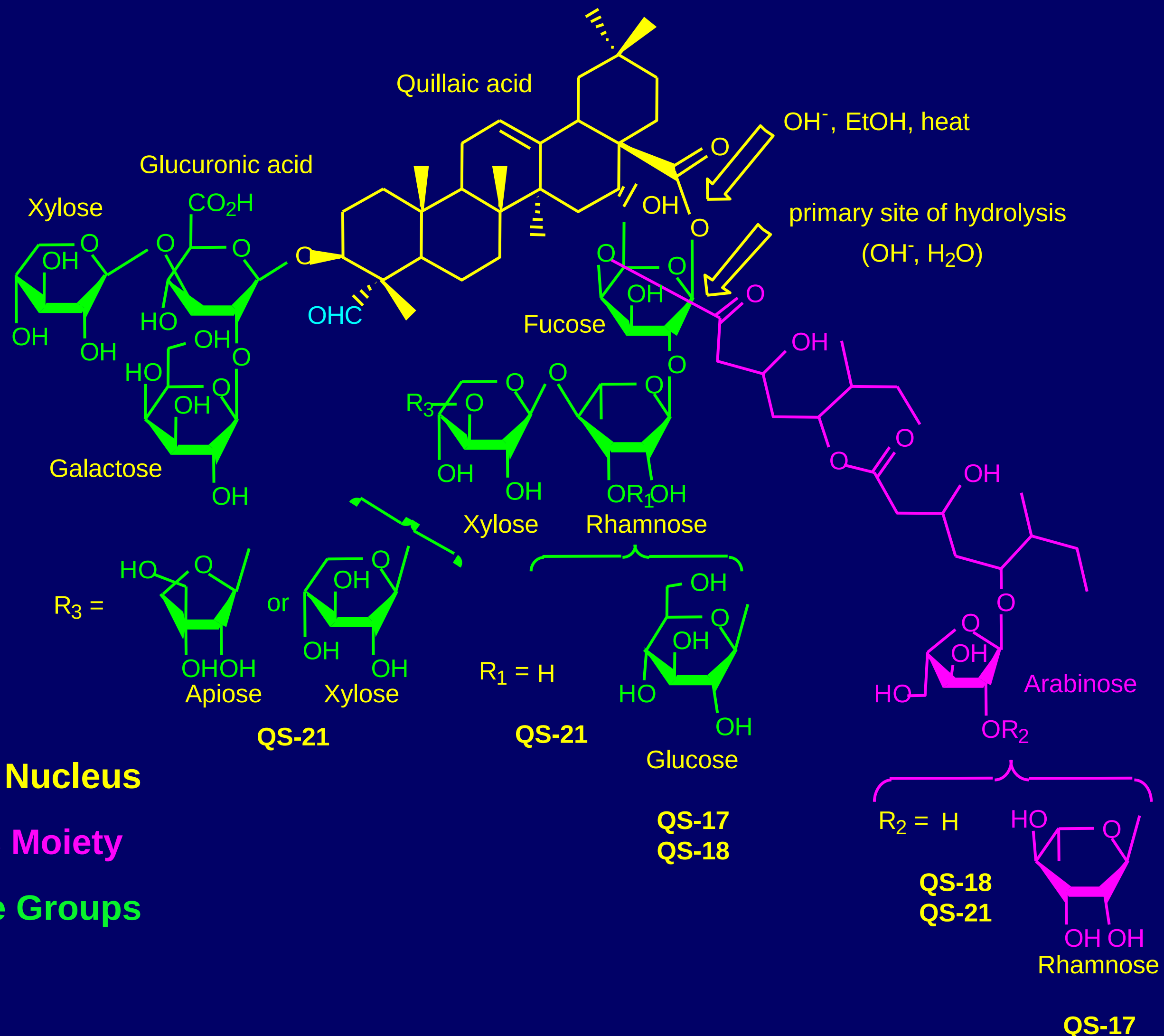
Vaccine	Adjuvant	Dose	Median Titer	
			IgM	IgG
GM2-KLH	none	—	1/80	0
GM2-KLH	Detox	250μg CWS/25 μg MPL	1/160	0
GM2-KLH	BCG	10^7 viable units	1/320	0
GM2-KLH	QS-21	10μg	1/320	0
	QS-21	50μg	1/320	1/40
	QS-21	100μg	1/1280	1/320
	QS-21	200μg	1/1280	1/640

ELISPOT Reactivity in Melanoma Patients Immunized With Tyrosinase 368-376 (370B) Peptide plus IFA (Montanide ISA-51), QS-21 or GM-CSF



Schaed, S.G., Chapman, P., et al, Clin Ca Res 967, 2002

Adjuvant Active Components of Quillaja Saponins



Triterpene Nucleus

Lipophilic Moiety

Saccharide Groups

GPI

Q. Saponins & Analogs Proposed Mechanism for Adjuvant Effect

- Saponin sugar moieties target the delivery of the saponin-Ag complex to APCs by binding to their cell surface lectins
- Saponin aldehyde forms an imine group with certain T-cell surface -NH2 groups & provides a costimulatory signal, replacing the B7-1 ligand and biasing the immune system toward a Th1 response

GPI

Q. Saponins & Analogs Proposed Mechanism for Adjuvant Effect

- Q. saponins and their derivatives having a hydrophobic chain allow the processing of exogenous proteins by the endogenous pathway
- Combination of the costimulatory signal and endogenous process results in the production of Ag specific CTLs

GPI

Impact of Immunological Adjuvant on Immunogenicity

MICE

- IFNγ release: KLH 180 $\rightarrow$ 14,560 pg/ml
- Antibody titers: GD3: 0 $\rightarrow$ 1/12,150

  MUC1: 1/100 $\rightarrow$ 1/3X10^6

  KLH: 1/160 $\rightarrow$ 1/7290

HUMANS

Antibody titers: GM2: 1/80 $\rightarrow$ 1/1280

Ways to Augment Vaccine Immunogenicity

- Modify the antigenic epitope
- Modify nearby components
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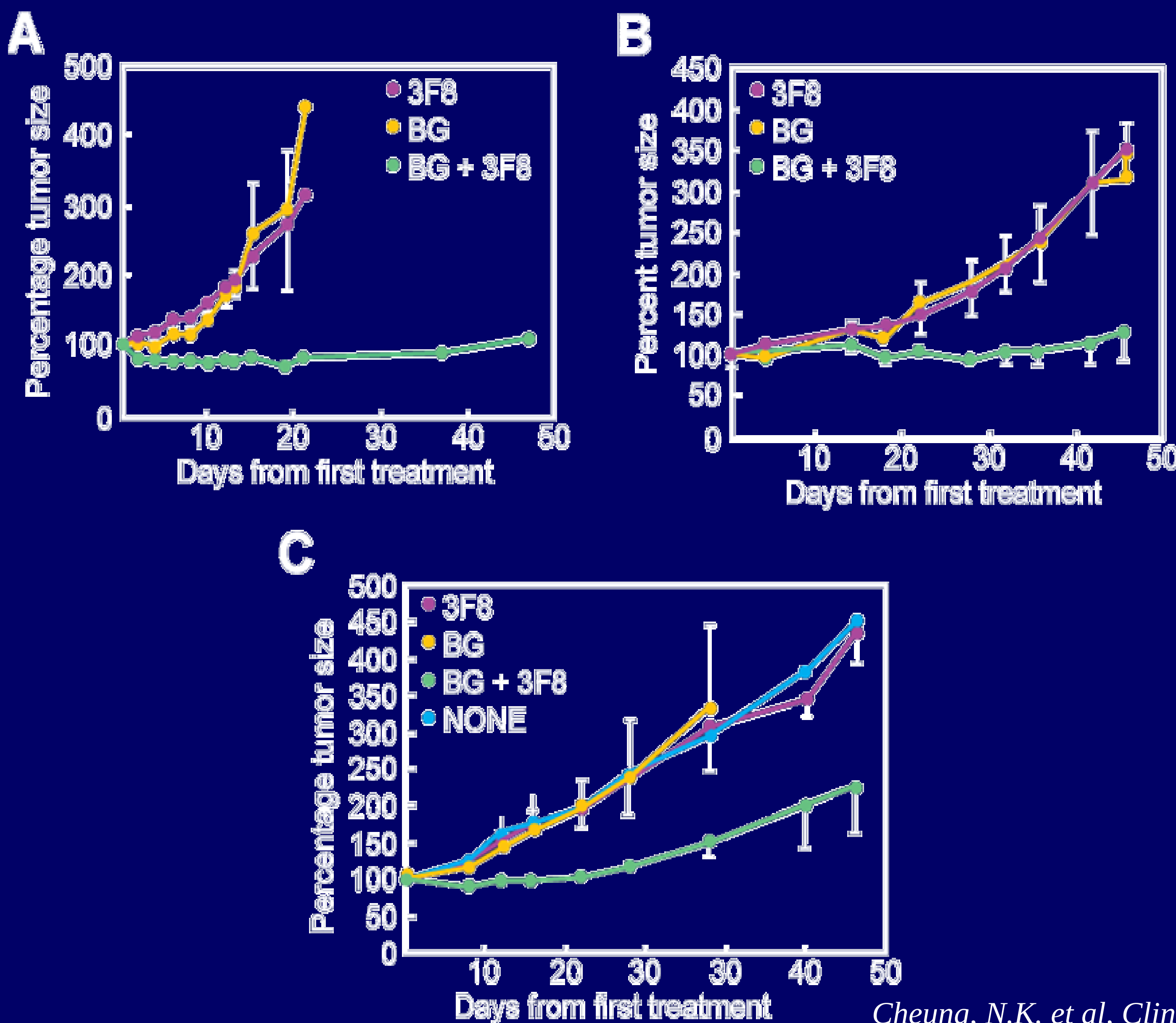
- Immune modulators

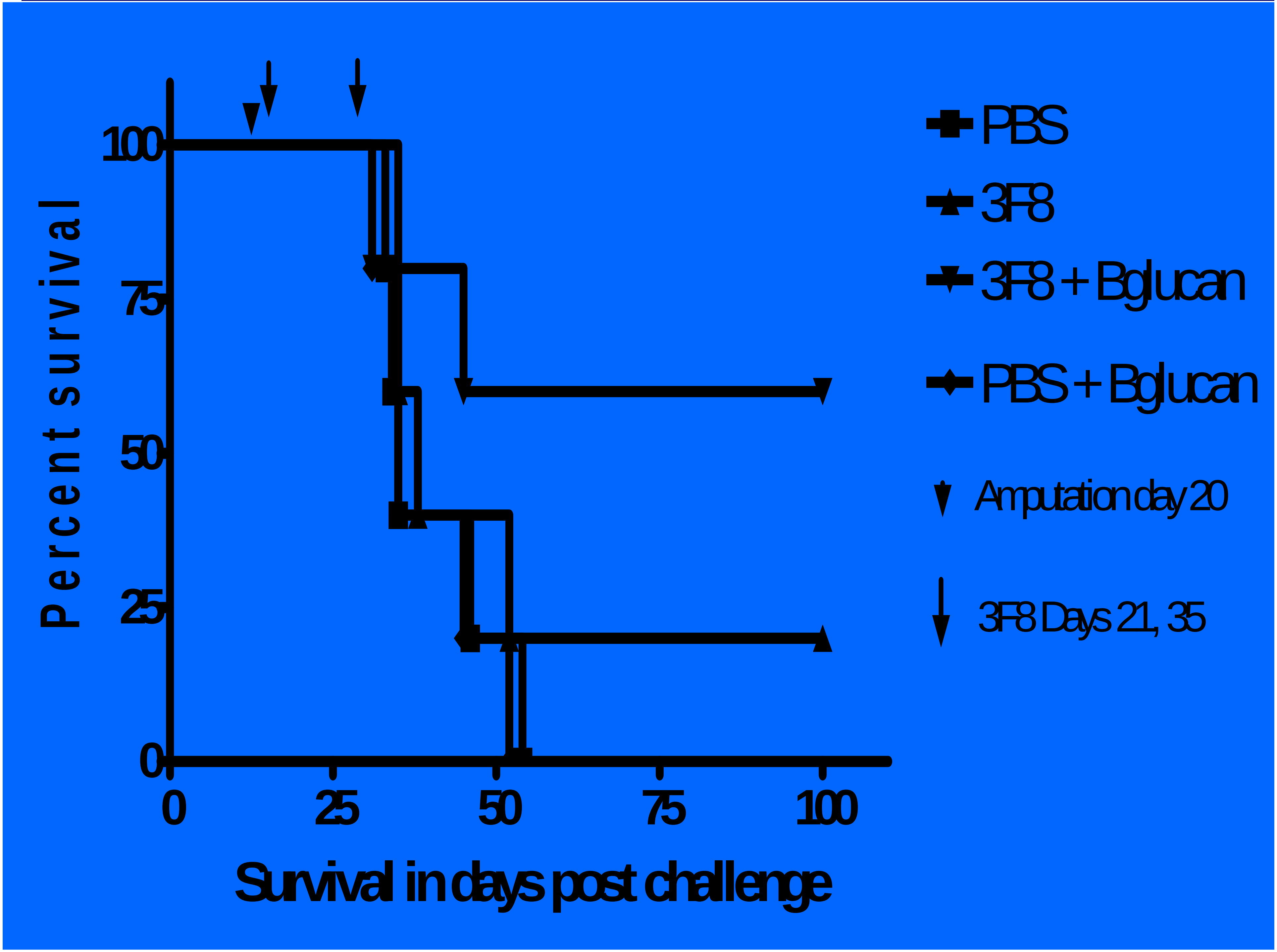
  Decrease T-regulator cells (Cytoxan, ↑CTLA4)

  Increase effector mechanisms

Synergistic Effect of IV 3F8 mAb and PO ß-glucan (BG) on Growth of 3 Human Neuroblastoma Cell Lines in Athymic Mice



Cheung, N.K. et al, Clin Ca Res in press



Percent survival
100
75
50
25
0
0
25
50
75
100
Survival in days post challenge
PBS
3F8
3F8 + Bglucan
PBS + Bglucan
Amputation day 20
3F8 Days 21, 35

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