Virus Like Particles as vaccine antigens and immunopotenciators

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Why to use Virus Like Particles?

- **Particles size lower than 50 nm in addition to humoral response are more able to promote CD4 and CD8 type 1 T cell response. This property made VLPs useful for therapeutic vaccination.**
- **VLPs utilizes the principle of ordered and repetitive antigen presentation to overcome B-cells tolerance, inducing auto-antibodies to self-molecules that mediate disease (cancer, allergy, and others).**
- **Particulate antigens could be presented to the immune system through B cells.**
- **VLPs usually facilitate the right antigen conformation.**
- **Cross-presentation of VLP antigens occurs much more efficiently than cross-presentation of soluble antigens.**
- **VLPs are only processed by professional APCs and not by other cell types such as fibroblasts or T-cells that can turn off T cell responses.**

Rationality for using IN administration route with VLP

- Mucosal immune system is only activated by mucosal route and includes around 80% of immunocytes.

- Mucosal immune system doesn’t depress at the same speed compared to systemic compartments.

- Viral and subviral particles has been detected in mucosal secretions and associated organs.

- Mucosal tissues are adapted to induce immune responses to particulated antigens.

Mucosal mechanisms for Ag uptake: M Cells

M Cells: specialized cells for VLP uptake

(Neutra M. Ann Rev Immunol, 2000)

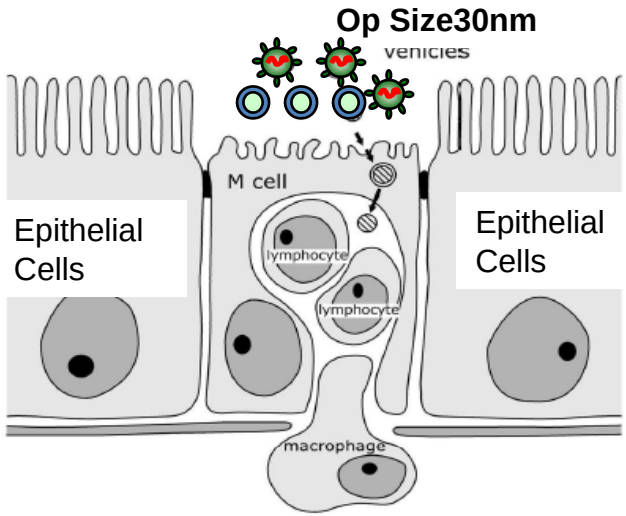
1- Lack of brush border organization

2- Basolateral pocket

3- Capacity for antigen endocytosis

4- Rapid transitosis w/o degradation

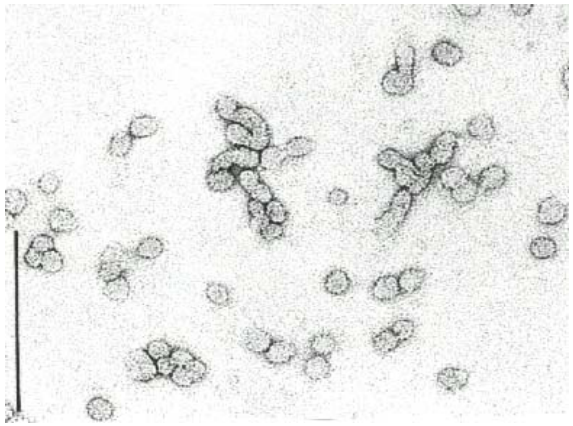
5- APC in the basolateral pocket



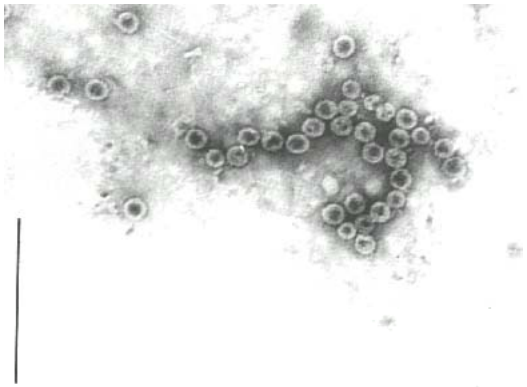
Hypothesis of the NASVAC project

Due to the physico-chemical and immunological properties , CIGB recombinant surface and nucleocapsid antigens could be used as immunogens as well as adjuvants in novel vaccine formulations administered by mucosal and parenteral routes

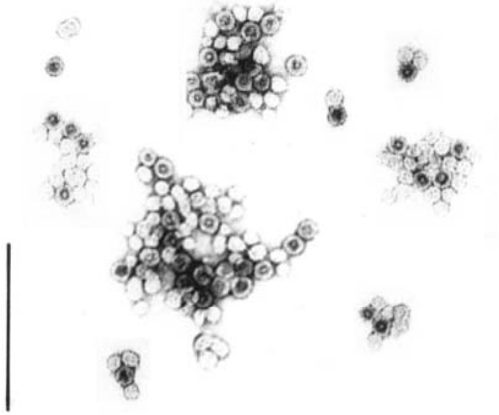
HBsAg



HBcAg



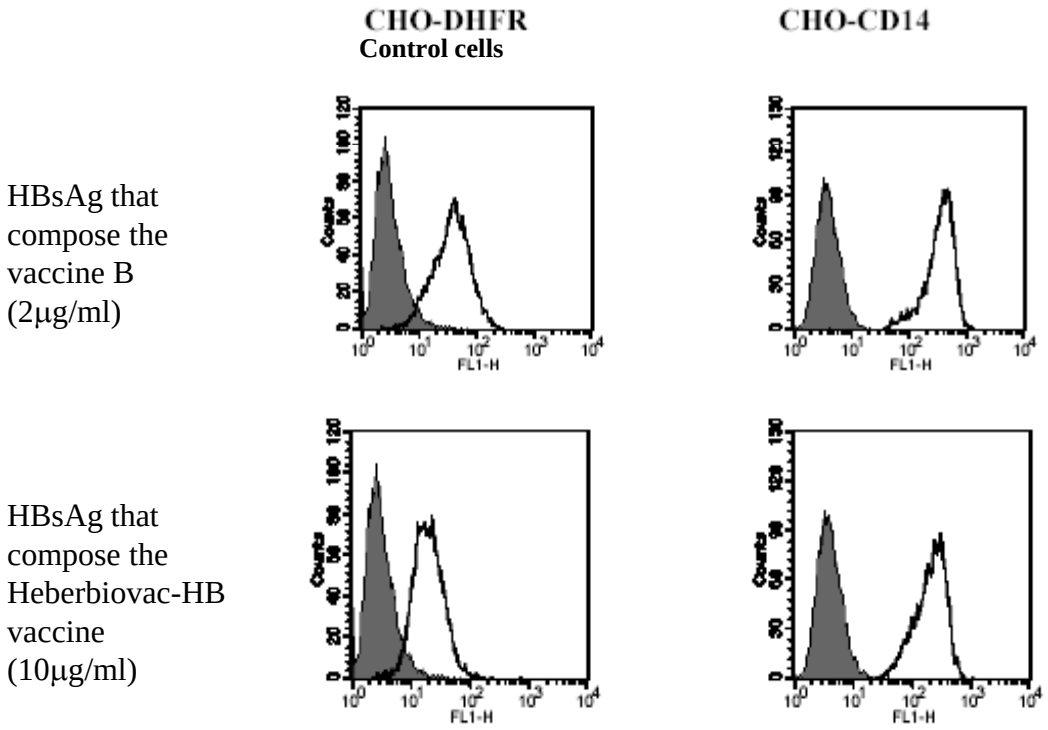
HBsAg + HBcAg



Bar: 200nm

- 20 to 30 nm particles and aggregates

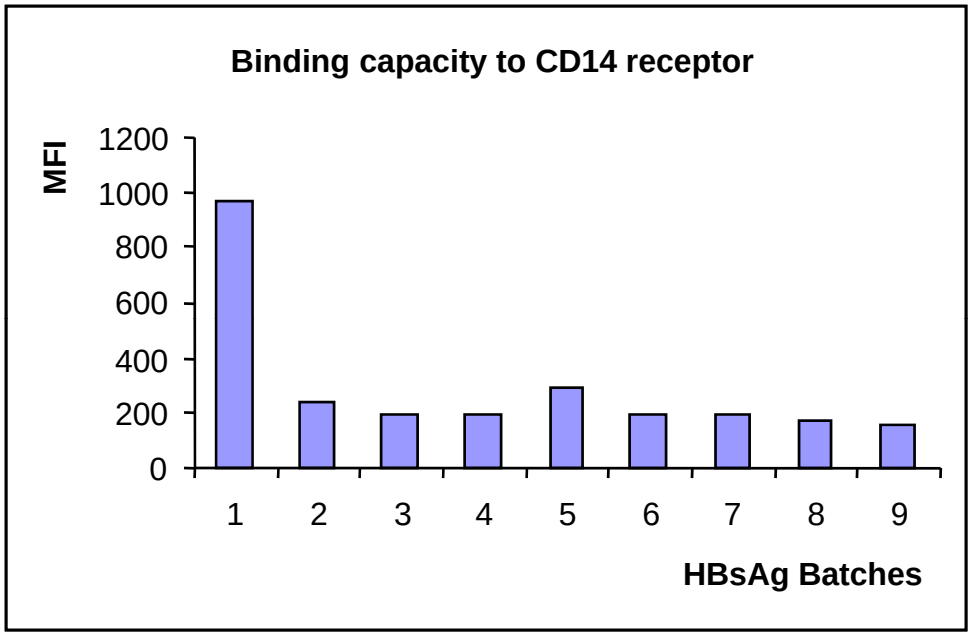
CD14 binding capacity experiments



✓ Binding of rHBsAg that compose the vaccine B to the CHO-DHFR control cells was detected, however, binding was strongly enhanced when CHO cells expressed CD14 were used. Attachment of rHBsAg to CHO-DHFR and CHO-CD14 cells was detectable too. However, 5 times more material was needed in the case of CIGB´s rHBsAg to obtained a similar CD14-binding pattern.

CD14 binding capacity experiments

FACS analysis of binding of HBsAg to CD14



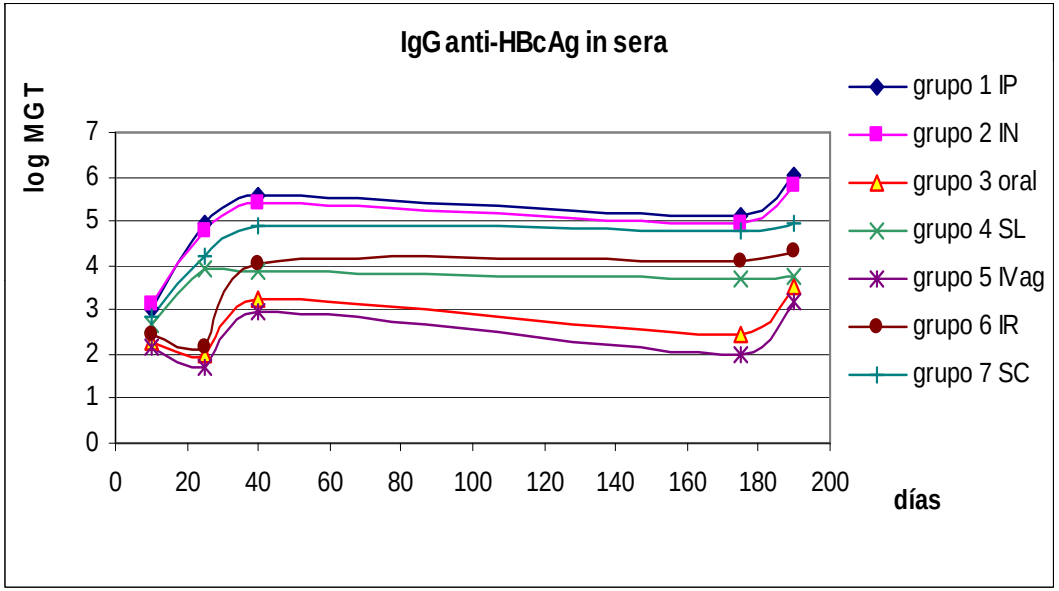
group 1) HBsAg that compose the vaccine B (produced in *Saccharomyces cerevisae*)

groups 2-9) HBsAg that compose the Heberbiovac-HB vaccine (produced *in Pichia pastoris* at the CIGB facilities)

✓ We detected that rHBsAg expressed in *Pichia pastoris* contains phosphatidyl serine (PS). This lipid is absent in *Saccharomyces cerevisae*-expressed HBsAg. This differential lipid content could explain the different pattern of binding of the rHBsAg to the CD14 membrane receptor.

Peter Vanlandschoot and Geert Leroux-Roels . Ghent University Hospital

The immunogenicity of HBcAg is optimal by IN route

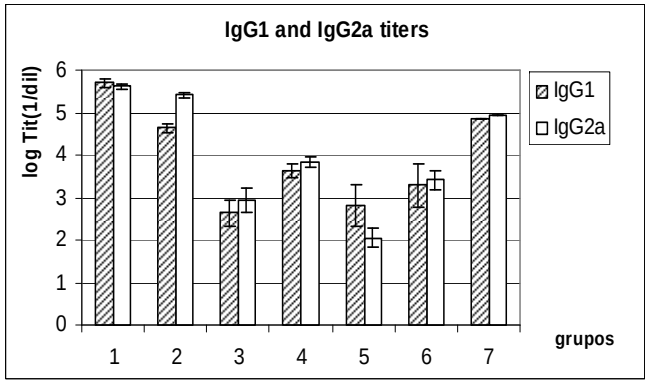


Schedule: (Balb/C mice)

Inoc: Days 0, 15, 30, 180

Extr: Days 10, 25, 40, 190

Dose: 5 µg HBcAg

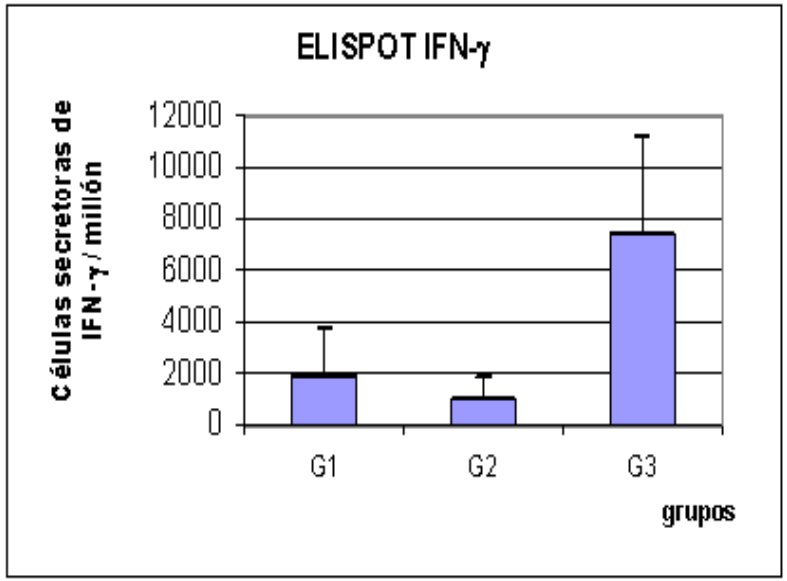


- HBcAg: a highly immunogenic antigen by parenteral and mucosal routes

- The IN route was optimal mucosal route for HBcAg immunization according to intensity and immunomodulatory aspects.

NASVAC and enhancement of Th1 immune response

ELISPOT IFN-g after estimulation with peptide CTL S (28-39)



Groups

G1: 5ug HBsAg in PBS, IN

G2: 5ug HBsAg in AlOH, IM

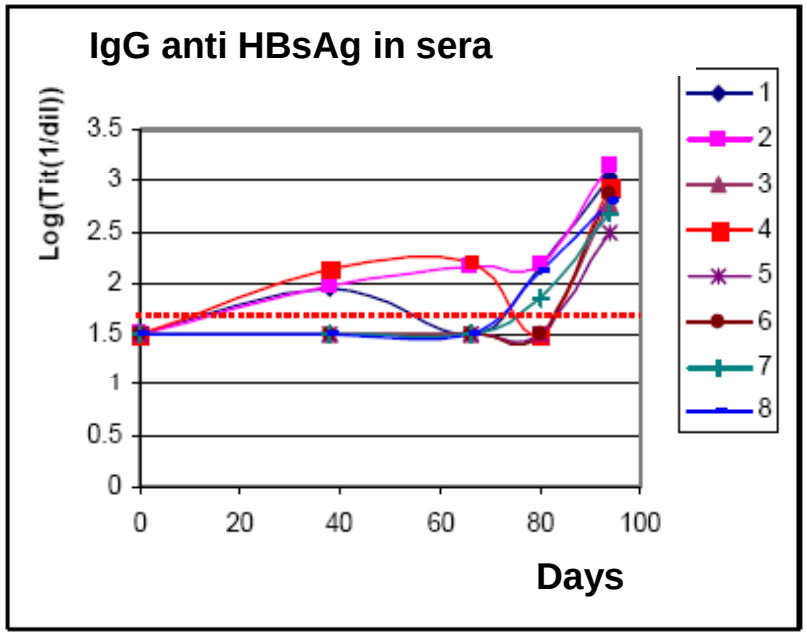
G3: ug HBsAg/5ug HBcAg, IN

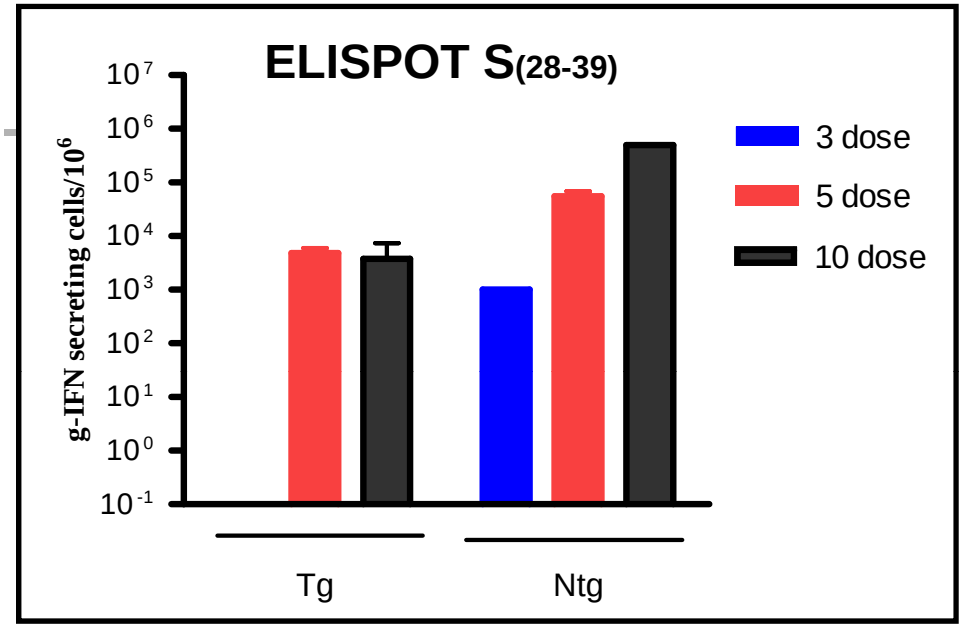
G3 vs G1 y G2**

Elispot assay carried out on day 120 after the booster dose (day 90), n=5

A significant increase in IFN-g secreting cells was obtained by inmunization with the combined formulation

NASVAC studies in HBsAg transgenic mice





- Humoral response against HBsAg detectable in most of tg mice after five-seven dosis.

- NASVAC induced a cellular immune response against HBsAg in HBsAg tg mice.

Phase I-II Clinical Trial at Lab Aid Hospital- Bangladesh Immunological response at Ehime Hospital -Japan)

Production of cytokines in sera after first cicle (by Cytometric Bead Array)

Names of cytokines	N	Before vaccination	After 5 IN vaccinations	p
IL8	20	23.7±4.7	96.1±29.5*	<0.05
IL-1 beta	20	5.4±0.45	7.3±0.83*	<0.05
IL-6	20	6±0.55	8.5±1.2*	<0.05
TNF-alpha	20	5.35±0.85	6.16±0.53*	<0.05

13 chronic patients HBeAg (-) / 7 HBeAg (+)

Month 0 1st cicle 5 IN administrations every 14 days

100 ug HBsAg + 100 ug HBcAg

Month 6 2nd cicle IN / SC 5 administrations every 14 days

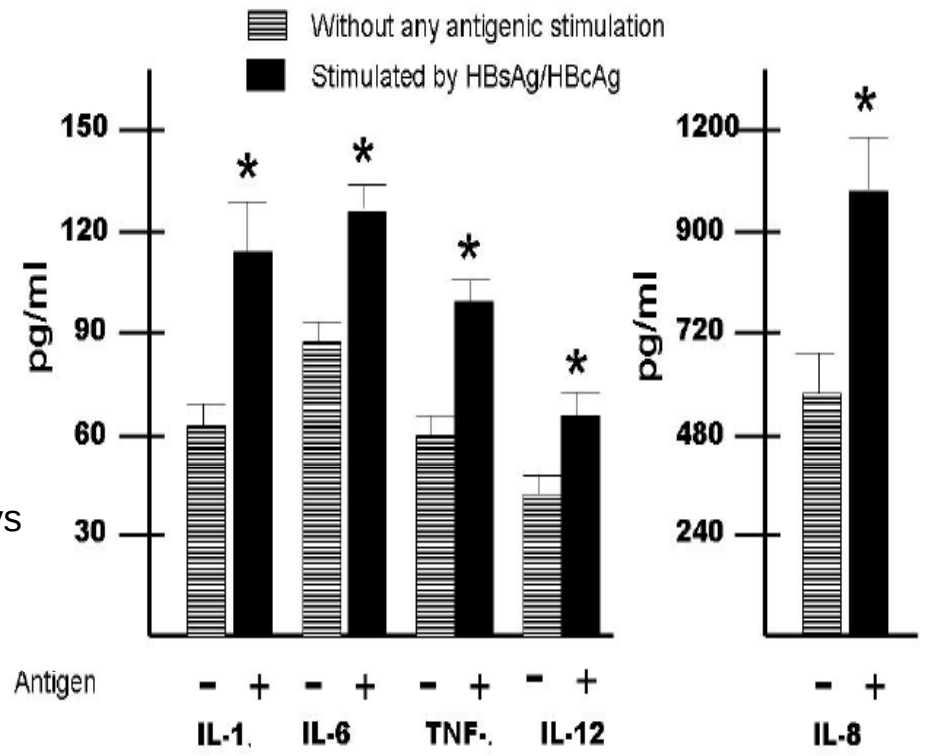
100 ug HBsAg + 100 ug HBcAg (IN)

100 ug HBsAg + 100 ug HBcAg (SC)

6 of 13 HBeAg (-) eliminate the virus

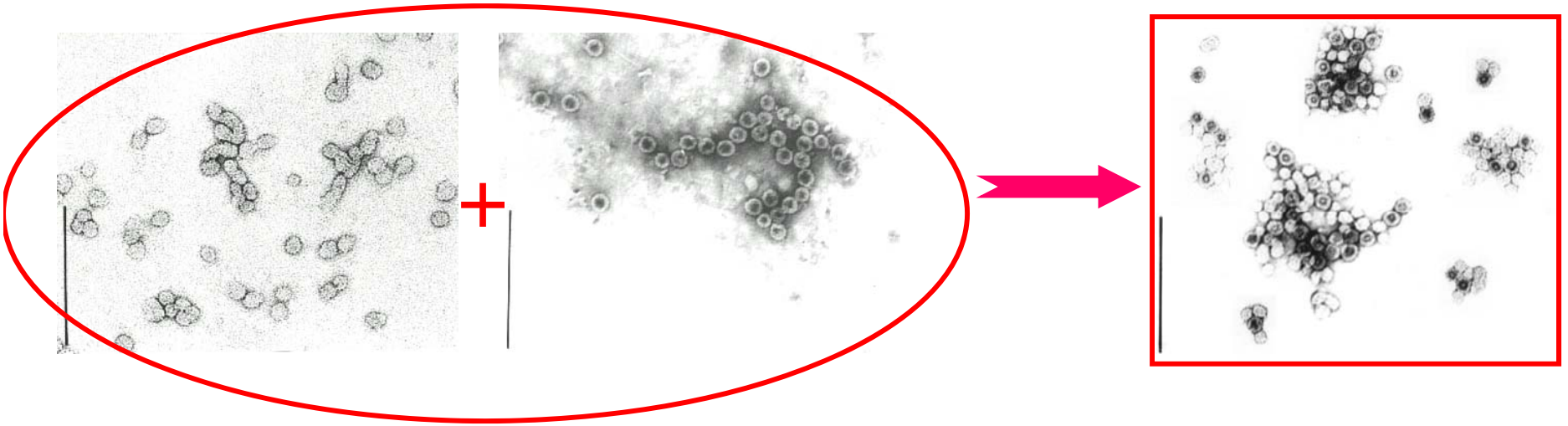
1 of 7 HBeAg (+) eliminate the virus

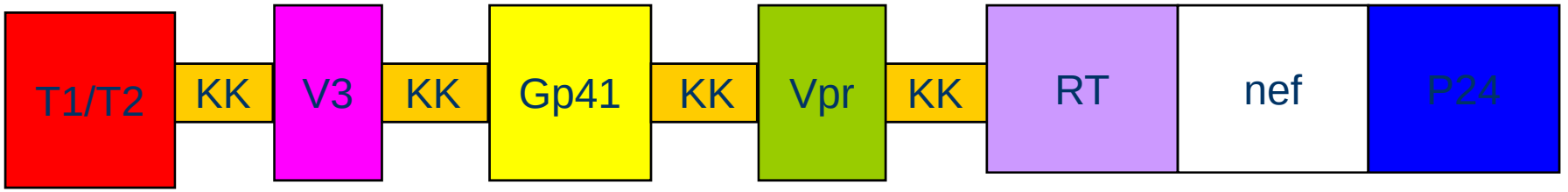
Compilation of data of cytokine production by PBMC of CHB patients vaccination with HBsAg/HBcAg



Development of a therapeutic vaccine candidate MEP-CR3 against HIV

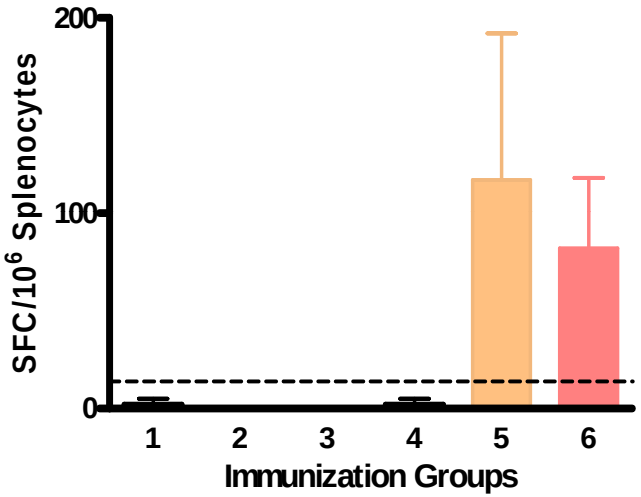
- ✓ MEP-CR3 is formulated with the mixture of
  HB surface antigen + HB core antigen



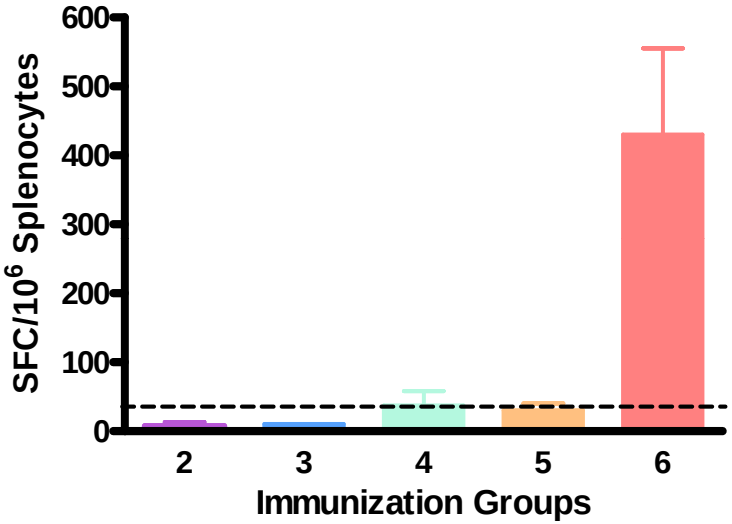


ELISPOT IFN-γ anti-CR3

Exp 1 (i.n.)



Exp 2 (i.p.)



--------- Threshold

- **Exp 1**

1. Placebo (PBS)
2. HBcAg + HBsAg
3. CR3
4. HBcAg + CR3
5. HBsAg + CR3
6. HBcAg + HBsAg + CR3

- **Exp 2**

1. Placebo (PBS)
2. HBcAg + HBsAg
3. CR3
4. HBcAg + CR3
5. HBsAg + CR3
6. HBcAg + HBsAg + CR3

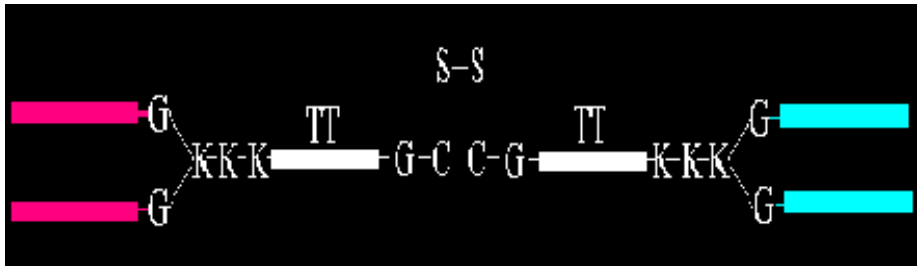
#	aa sequences	1	2	3	4
1	SVHMGPGRAFYATGDIIGDIRQ				
2	SVHMGPGKTFYATGDIIGDIRQ				
3	SMRIGPGQTFYATGDIIGDIRQ				
4	SIRIGPGQAFYATGDIIGDIRQ				
5	SIHIAPRQAFYATGAIIGDIRQ				
6	SVRIGPGQAFYATGDIIGDIRQ				
7	SVNIGPGQAFYATGDIIGDIRQ				
8	GVHIGPGQAFYARGDIIGDIRQ				
9	SLRIGPGQTFYATGDIIGDIRQ				
10	SVHIGPGQAFYATGDIIGDIRQ				
11	SVRIGPGQTFYATGDIIGDIRQ				
12	GIHIGPGSAIYATGDIIGDIRQ				
13	GIHIGPGQTFYATGEIIGNIRQ				
14	SVRIGPGQTFYATGAIIGDIRQ				
15	SVRIGPGQTFYAAGDIIGDIRQ				
16	SVRIGPGQAFYATGDIIGDTRQ				
17	SIHFGPGQTLYATGNIIGDIRQ				
18	SIRIGSGQTSYATGDIIGNIRE				
19	GIHIGPGRAFYATGQITGDIRQ				
20	SIRIGPGQAFYATGDIIGNIRQ				
21	SIHIGPGQAFYATGDIIGNIRQ				
22	SIRIGPGQTFYATGDIIGNIRQ				
23	SIRIGPGQAFYATGDITGDIRQ				
24	SIHIGPGQAFYATSDIIGDIRQ				
25	SIRIGPGQAFYTTGDIIGDIRQ				
26	SVHIGPGQAFYATGDIIGNIRQ				
27	SANIGPGQAFYATGEIIGDIRQ				
28	GIHIGPGQSFYATGSIVGNIRQ				
29	SIRIGPGQTFYATGEIIGNIRQ				
30	GVHIGPGQAFYATGDIIGDIRQ				
31	SIRIGPGQSFHATGDIIGDIRQ				
32	SVHIGPGQAFYATGDVIGDIRQ				
33	GVRIGPGQAFYATGGIIGDIRQ				
34	SVHIGPGQTSYATGDIIGDIRQ				
35	SIHLGPGRAFYATGDIIGDIRQ				
36	SVRIGPGQAFYATGDIIGNIRQ				
37	SVHIGPGQAFYARGDIIGDIRQ				
38	SIHIGPGQAFYARGDIIGNIRQ				
39	SVHIGPGQAFYATGEIIGDIRQ				
40	SVRIGPGQTFYATGDIIGNIRQ				
41	SVHIGPGKAFYATGGIVGDIRQ				
42	SVHIGPGQAFYATGAIIGSIRQ				
43	SIGIGPGQTFYAADNIIGDIRQ				
44	SVRIGPGQSFYATGDIIGDIKQ				
45	SISIGPGRAFFATGDIIGDIRQ				
46	GIHMGPGQILYATGSIIGDIRQ				
47	SIRIGPGQVFYTNGDIIGDIRQ				
48	SIHIAPGQAFYATGAIIGDIRQ				
49	GIHLGPGQAFYATNAIIGDIRQ				
50	SIHIGPGQAFYATGDIIGDIRQ				

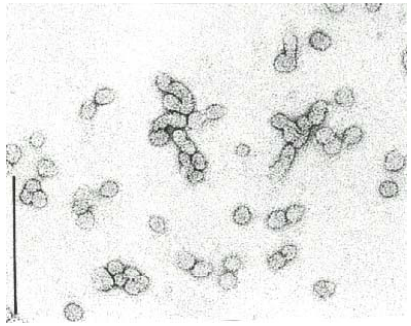
Crossreactive response of MAP conjugated to HBsAg VLP against HIV V3-Loop

Groups

1. HBsAg-consensus-MAP8
2. consensus- CD4bs-MAP4
3. HBsAg- consensus- CD4bs-MAP4 ***
4. HBsAg- CD4bs-MAP8

The crossreactive response was higher for the group containing the heterologous MAP





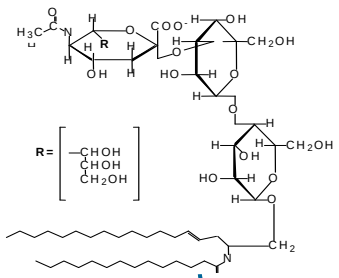
***Bioconjug Chem*. 2004. 15(1):112-20**

From *Neisseria meningitidis* to VSSP: a rational design of an adjuvant for Cancer Immunoterapy

This novel adjuvant is a nanoparticulated hydrophobic conjugate in which purified gangliosides were incorporated into the outer membrane protein complex of Nm to form Very Small Sized Proteoliposomes (VSSP)

Immunosuppresor shaded by tumor cells

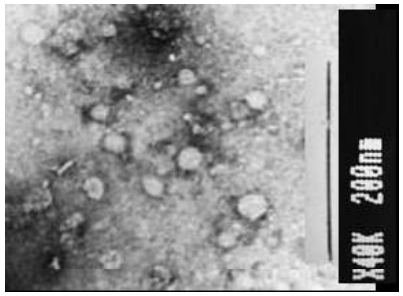
Gangliosides



Neisseria meningitidis

Strong Innate Immune ligand

VSSP

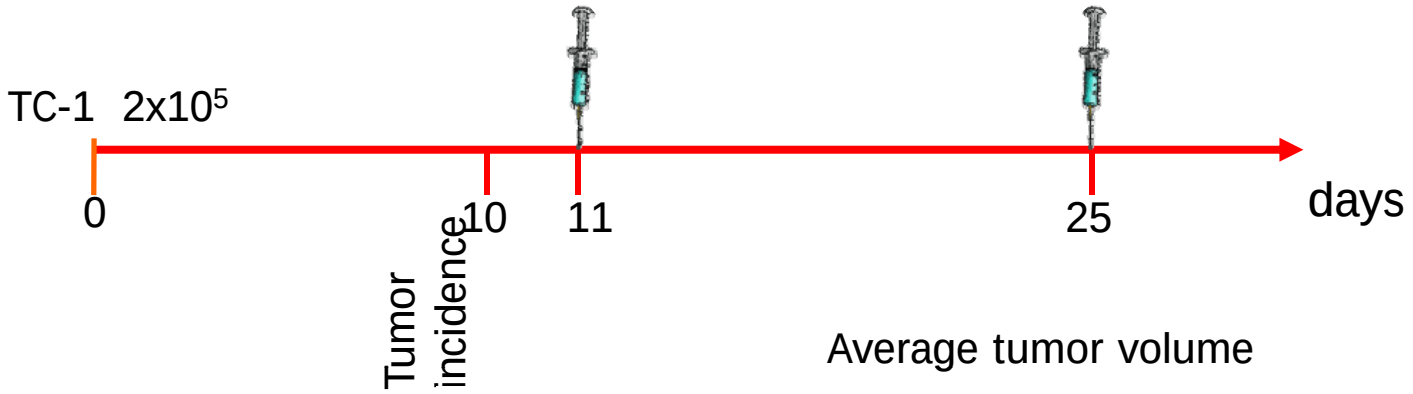


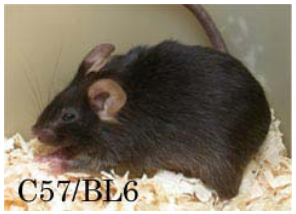
- Powerful danger signal
- CTL and antibody responses to peptides and proteins
- Dendritic cell activation
- Primary CD8 cell expansion without need of CD4+ help
- More than just LPS
- Ease of use: mix with antigen and inject s.c., i.m.

- Molecular Ratio 1:37 (P:G)
- Size 9 nm

Mesa C. *et al* Vaccine 2006

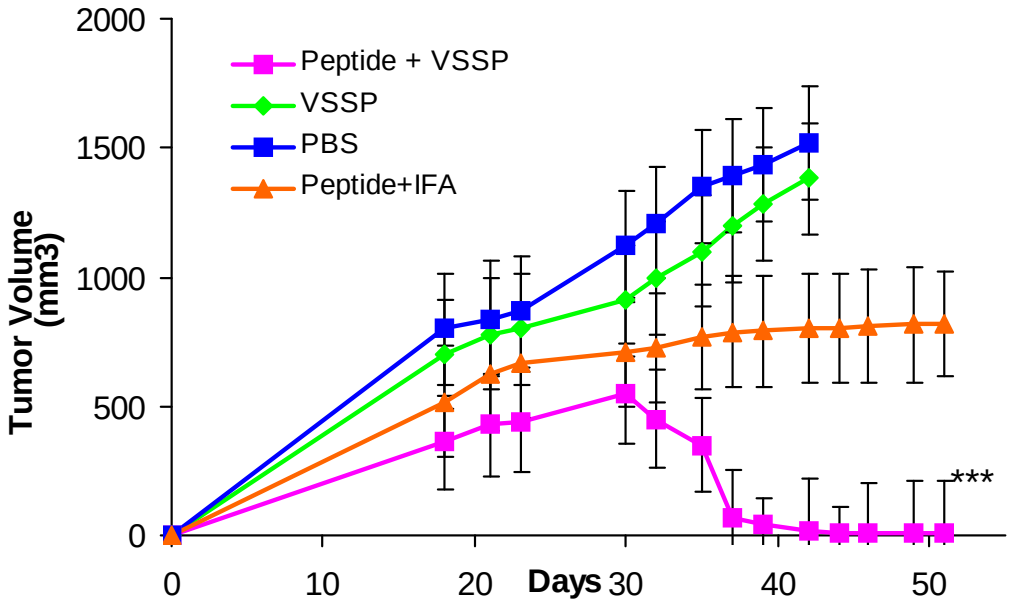
Therapeutic vaccine candidate against cervix cancer. Treatment of established tumors





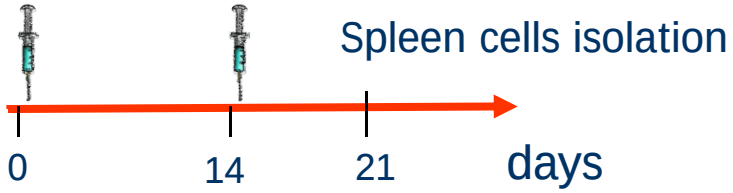
C57BL/6
6-8 weeks old
10 animals/ group
s.c. immunizations

Average tumor volume



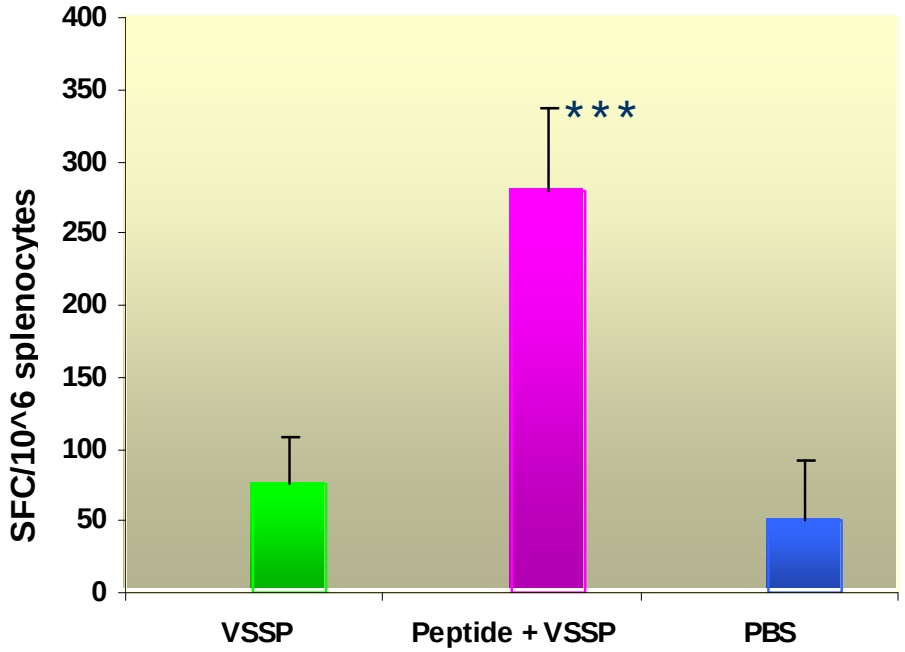
Mann- Whitney U test

Immunization is associated with IFN-γ production in spleen cells (ELISPOT)



C57Bl/6 mice
Spleen cells were *in vitro* sensitized with the CTL peptide from E7 HPV for 7 days

Target cell: EL4 + RAHYNIVTF



Summary of evaluations

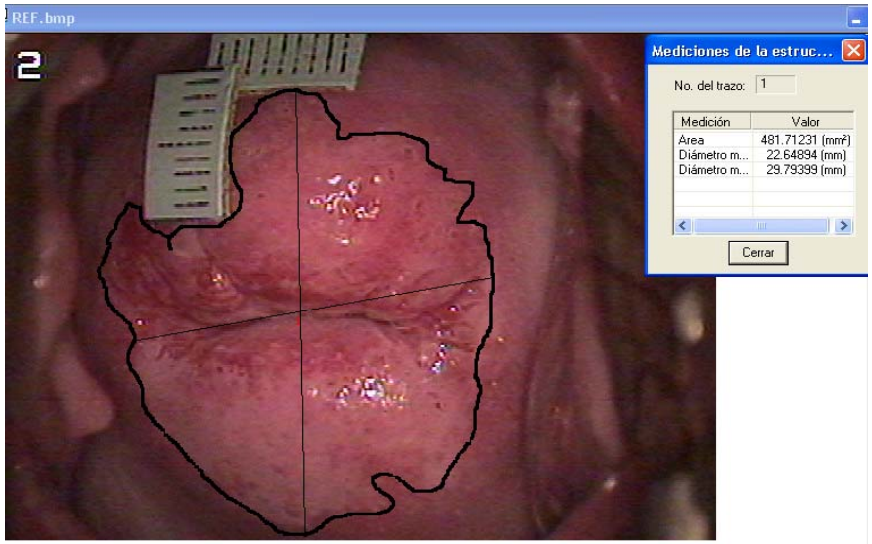
70% CR+PR in 3 months

Patient No	Colposcopy	Histology	Immunogenicity
01	CR	CR	+++
02	SD	SD	++
03	PR	PR	++
04	CR	CR	+++
05	CR	CR	++
06	CR	CR	+++
07	SD	SD	+

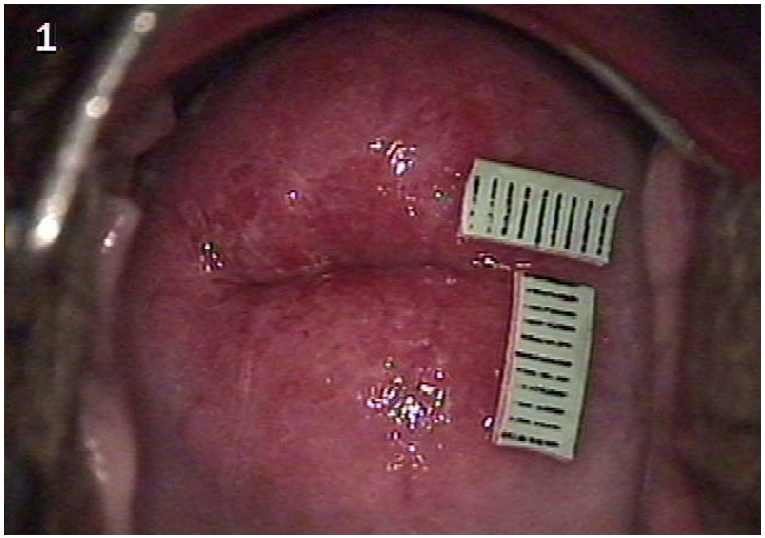
CR- complete response; PR-partial response; SD-stable disease

Pre-CIS

05



Post- RC (al 3er mes)



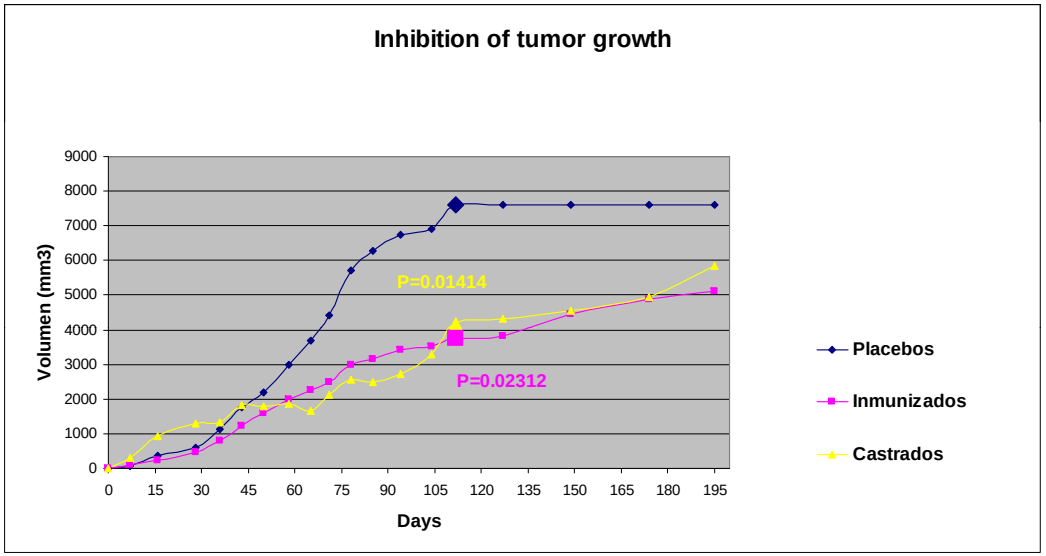
Pre-NIC III

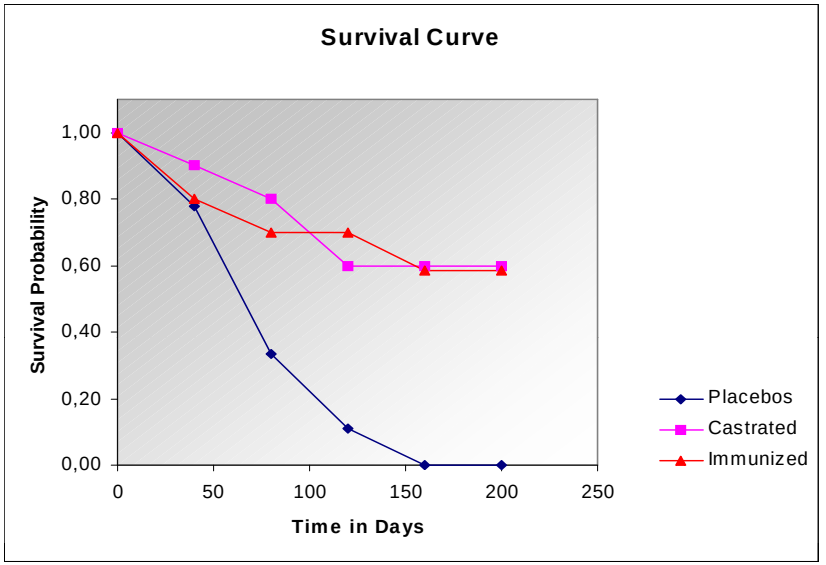
06

Post- RC (al mes)

Peptide vaccine candidate for hormone ablation in prostate cancer

Tumor growth rate and survival on Placebo, Castrated and Immunized rats.





Tumor Growth means volume of Placebo, Castrated and Immunized Copenhagen rats implated with The Dunning R3327-H prostate cancer cell line

Kaplan –Meier statistical analysis of survival probability of Immunized and Castrated rats compared with the placebo group

GnRHm1, 10 a.a.												T-helper epitope sequence from TT, 15 a.a.														
Q	H	W	S	Y	G	L	R	P	G	G	G	Q	Y	I	K	A	N	S	K	F	I	G	I	T	E	L

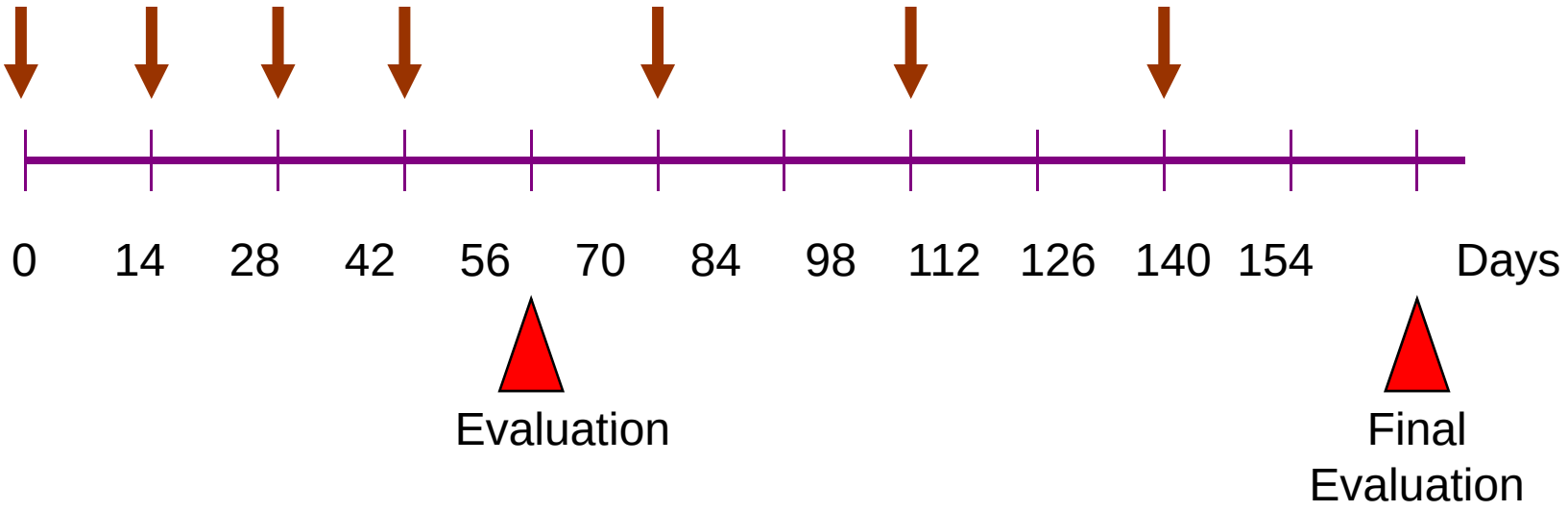
GnRHm1-TT, 27 a.a.

Prostate Cancer Vaccine: Heberprovac

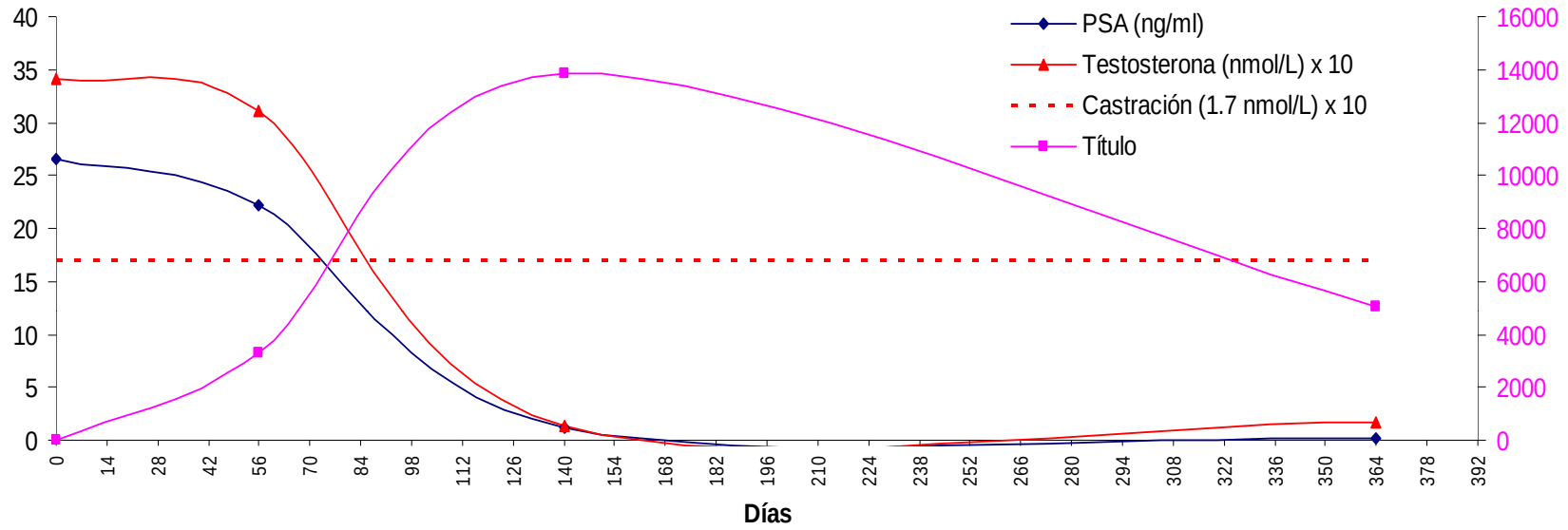
Components

- Peptide GnRHm1-TT (3mg).
- VSSP (245 µg)
- Montanide ISA-51 (350 µl)

Schedule:



Follow up of the clinical trial 25 month after the last immunization



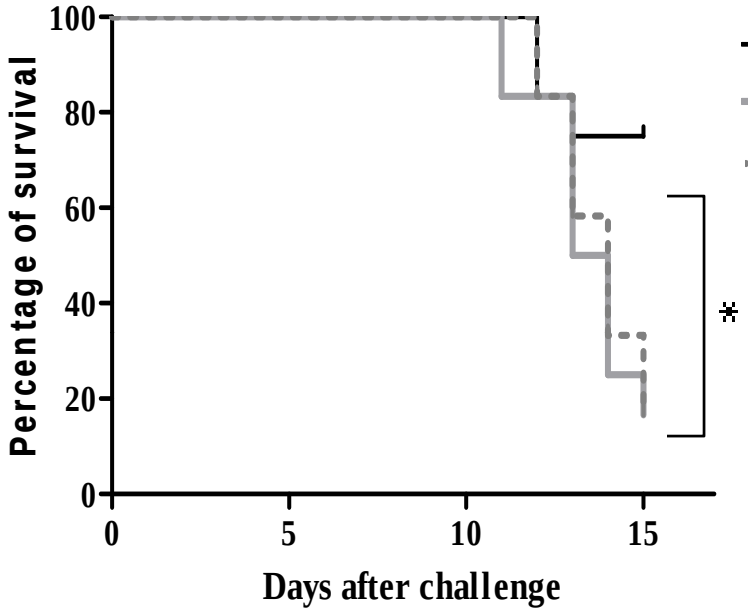
Digital rectal test:

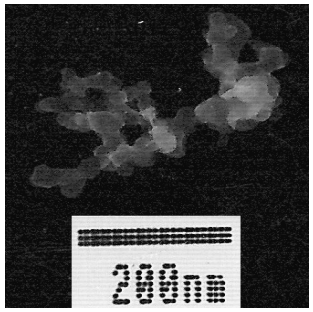
- **The 6 patients with normal prostate**
- **No serious urinary manifestations**

Hemochemical and hematological criteria:

- **Normal PSA in all the patients.**
- **Testosterone levels down the castration levels (1.75 nmol/L).**
- **Antibody titers anti GnRH in all the patients 1/50 - 1/100**

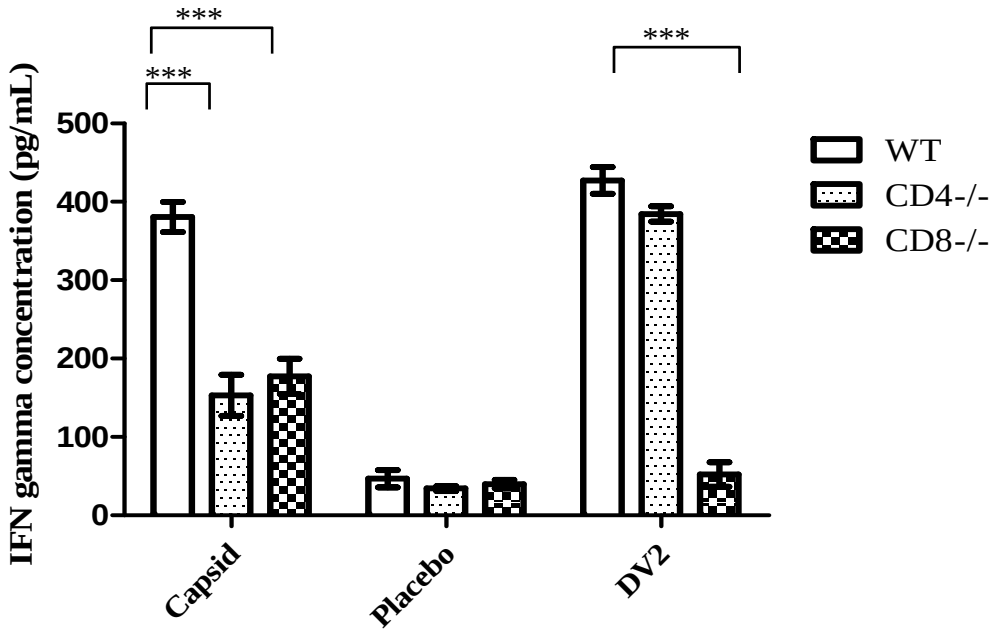
The highly purified and particulate dengue capsid protein protected mice against virus challenge and induced cell-mediated immunity





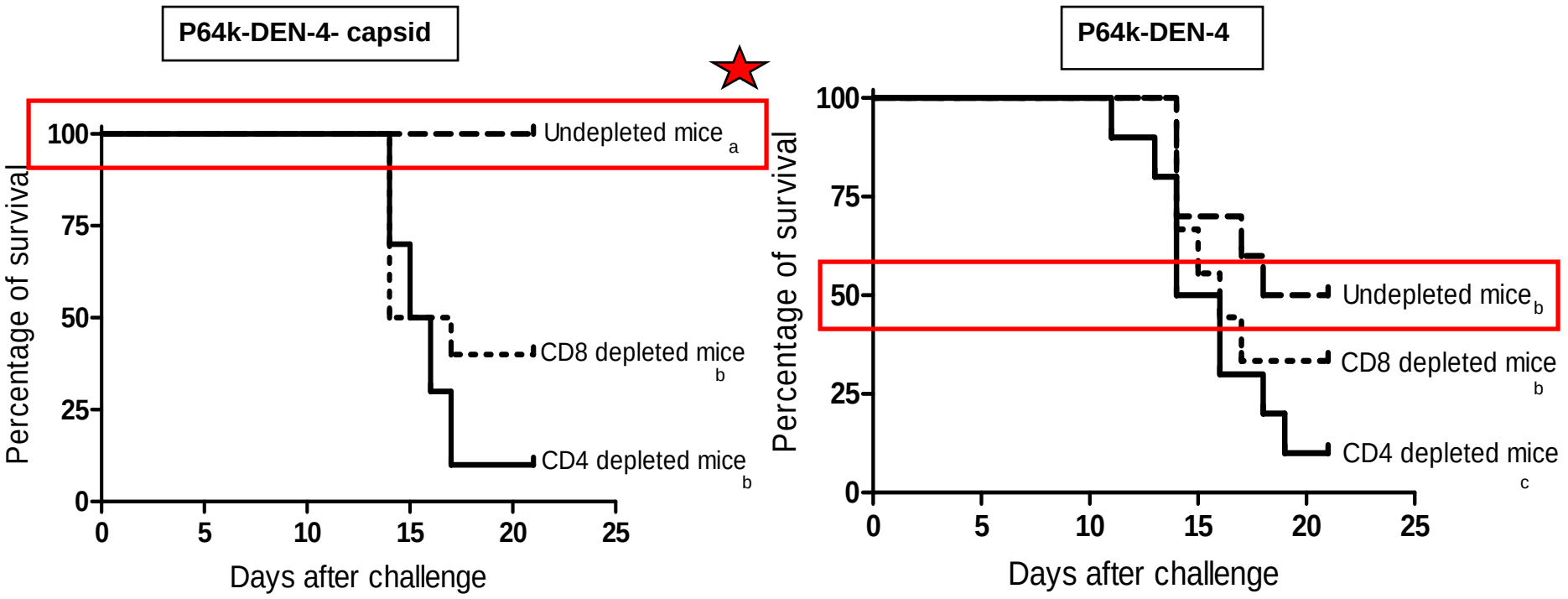
Capsid particulated antigen (30 nm) nanoparticles

In vitro stimulation with dengue-2 virus



Gil *et al*; International Immunology (in press)

The highly purified and particulate capsid protein improved the immunogenicity of the chimeric protein P64k-DEN-4. This effect was mediated by CD4+ and CD8+ cells



DEN-4 challenge

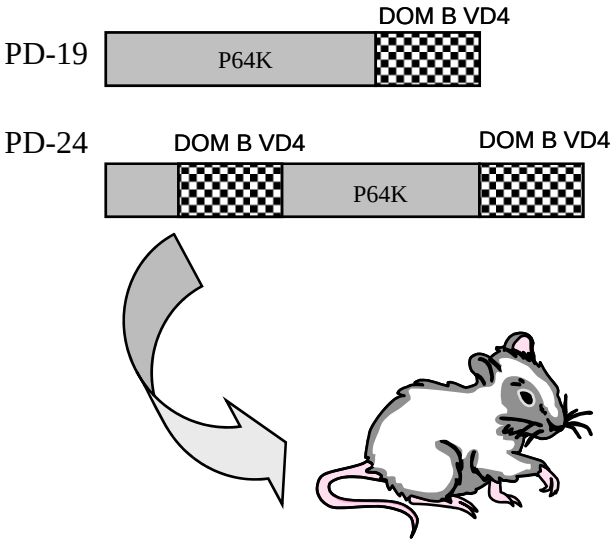
Lazo *et al*; data pending publication

Dengue vaccine candidate

Proof of concept with dengue vaccine candidate in Macacus irus

	0	1	2	3	4	5	6	7	8	9	10
P64k	-	-	-	+	+	+	-	-	-	-	-
4182	-	-	-	+	+	+	-	-	-	-	-
4196	-	-	-	+	+	+	-	-	-	-	-
T-21	-	-	-	+	+	+	-	-	-	-	-
PD5	-	-	-	-	-	-	-	-	-	-	-
4186	-	-	-	-	-	-	-	-	-	-	-
T-22	-	-	-	-	-	-	-	-	-	-	-
T-25	-	-	-	-	-	-	-	-	-	-	-

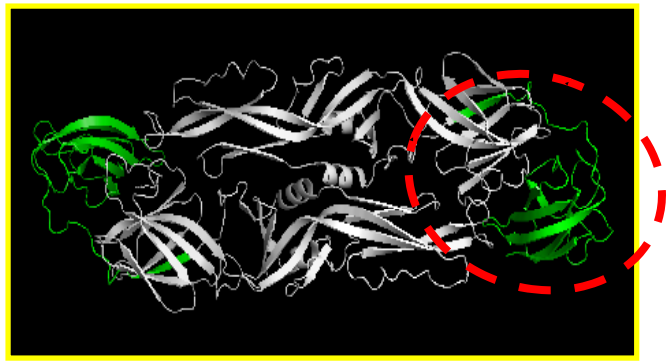
Formulation of dengue 4 Dom III chimeric protein with capsid protein from dengue 2

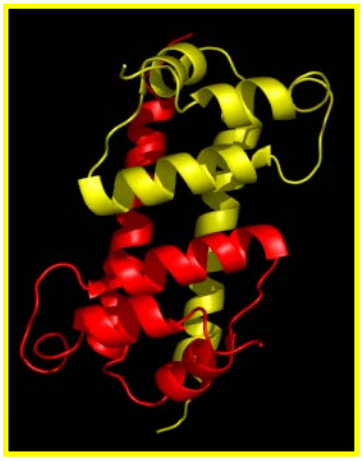


PD-24 D4	60%
PD-24 D4-core VD2	92%
PD-19 D4	40%
Core VD2	15%
Negative control	10%
Positive control VD4	90%

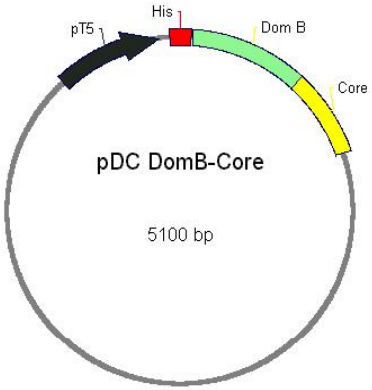
High level of protection for serotype 4

Dengue Chimeric capsid – Dom III proteins

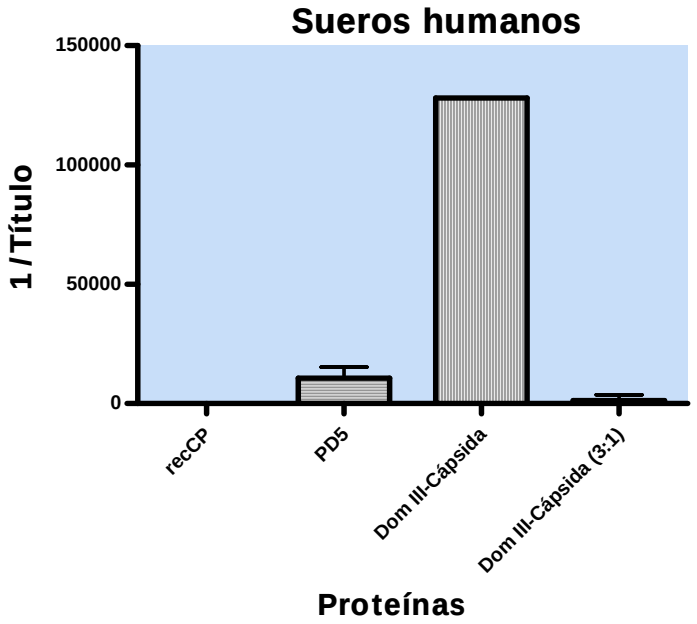




Dom III-Capsid



Properly recognized by positive human sera



1:160 000

MS Characterization of Dom III – Capsid chimeric protein with a right conformation

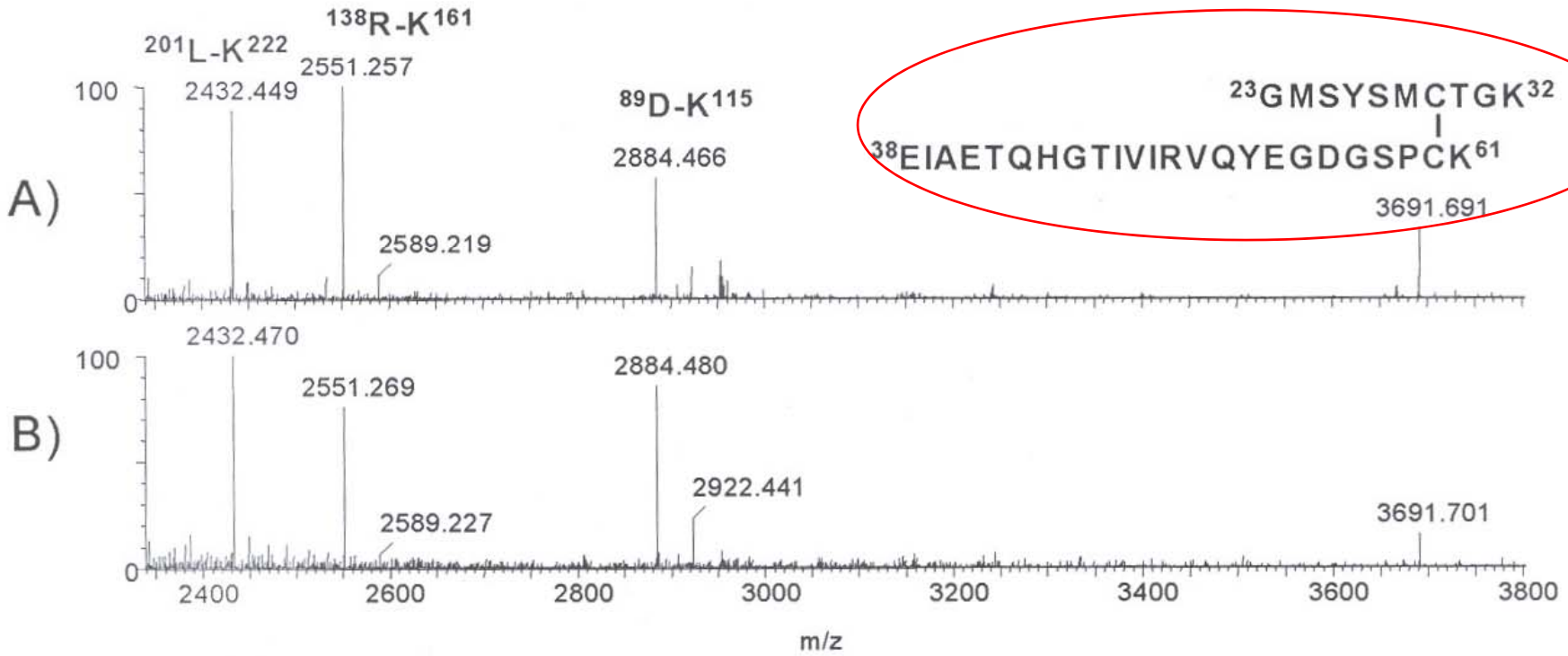
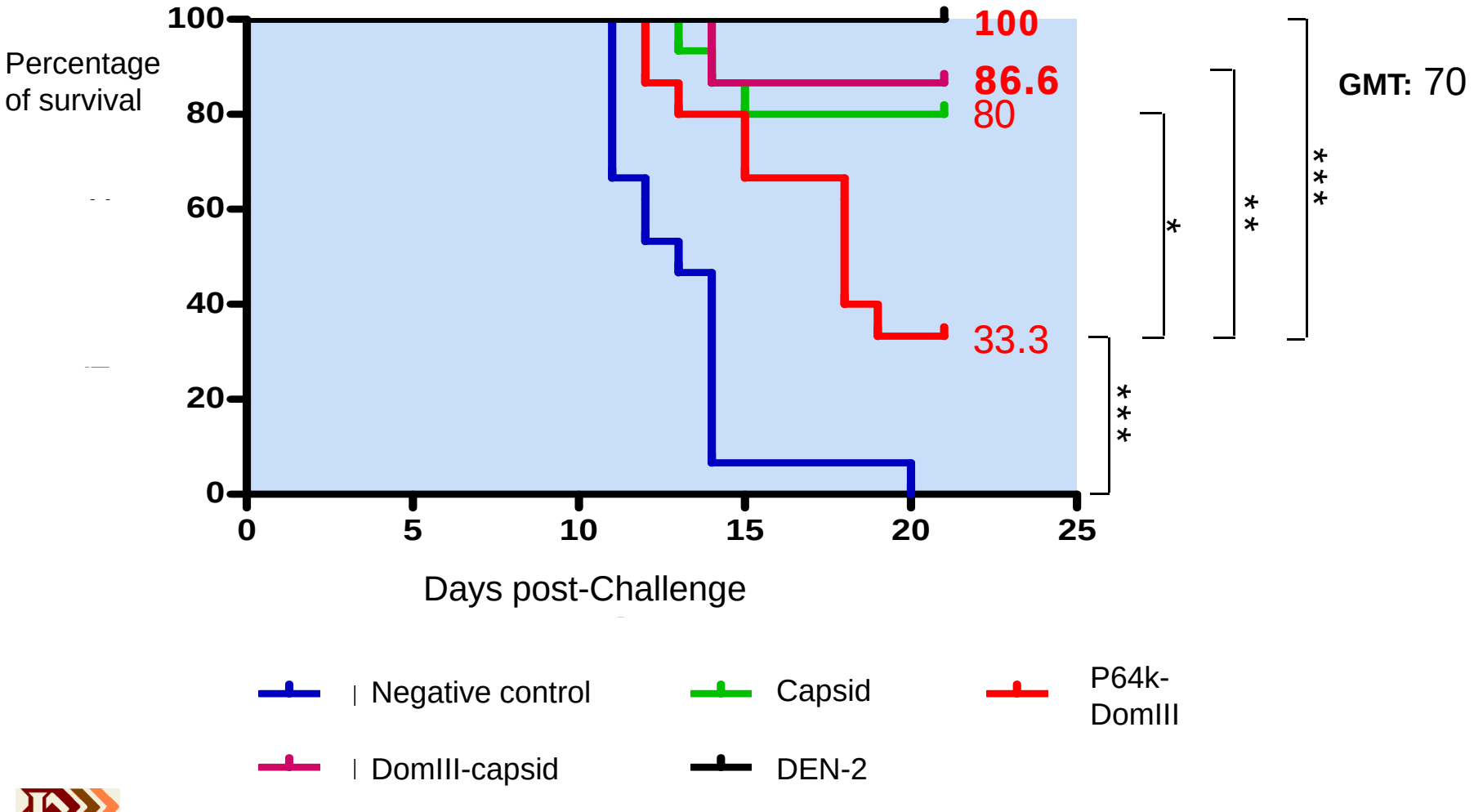


Figura 1. Rango parcial de los espectros ESI-MS deconvolucionados obtenidos de la digestión con LEP de la proteína nativa (A) y carbamidometilada (B). Se indican diferentes señales correspondientes a regiones de la proteína así como el puente de disulfuro entre las cisteínas 29 y 60.

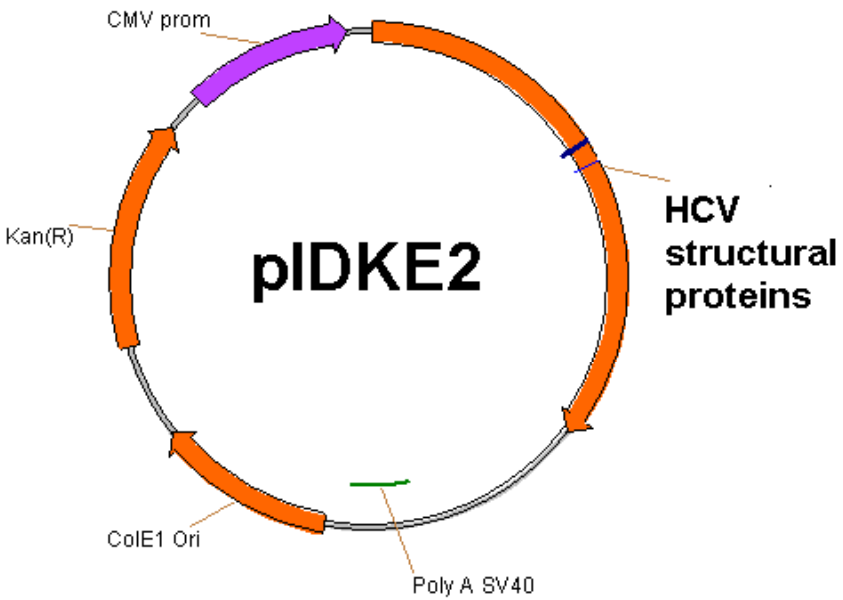
The chimeric protein DomIII-capsid protected mice against homologous virus challenge and induced neutralizing antibodies

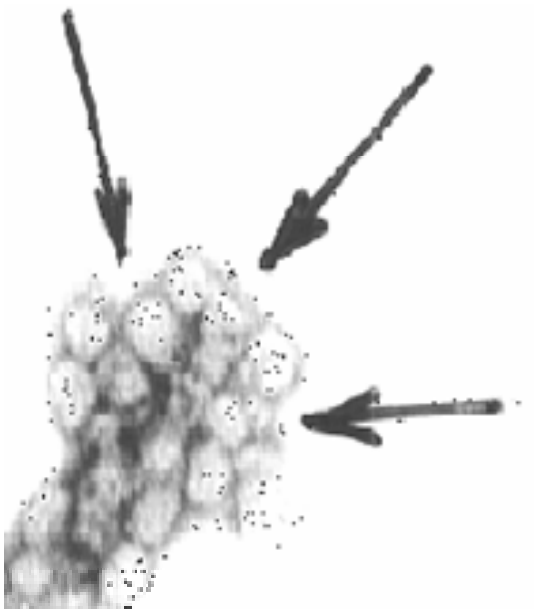


Valdés *et al;* data pending publication

Vaccine candidate against Hepatitis C

DNA construct + **Core particles**





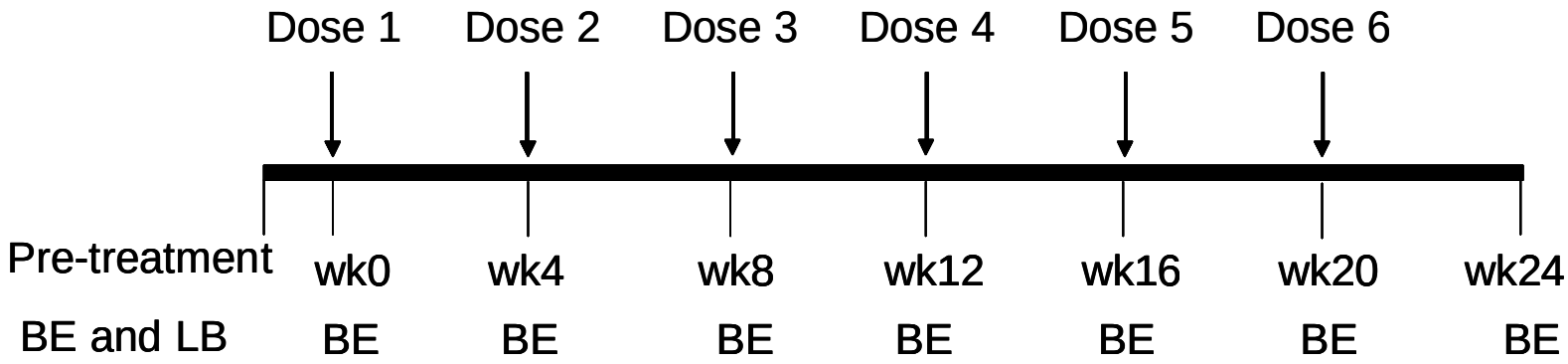
Biotechnol Appl Biochem. 2002;35:205-212

Vaccine 2001; 19:3940-3946

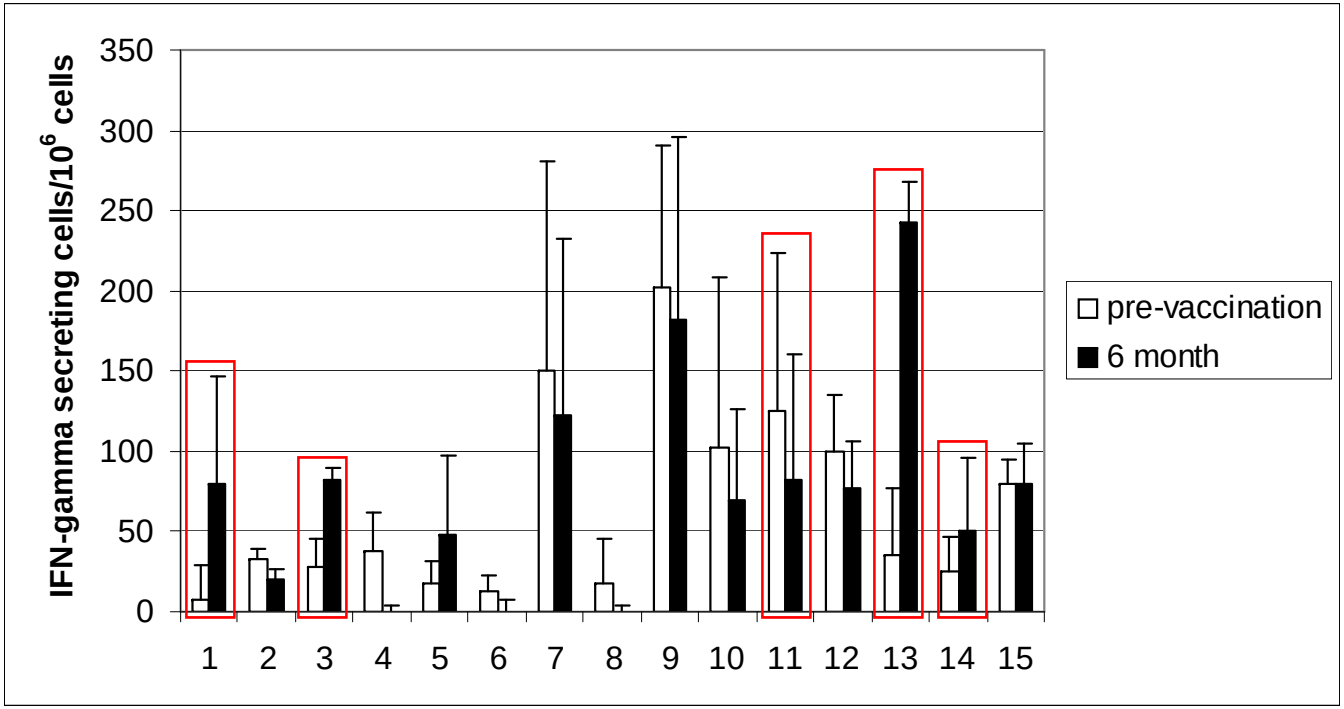
Phase I clinical trial in a difficult-to-treat group of patients

Preliminary evaluation of safety and pharmacodynamics of terap c: a therapeutic vaccine candidate for the treatment of chronic hepatitis c. a phase i clinical trial, not controlled, not randomized, not blinded

Code: IG/VHI/ HC/0503



IFN-gamma secretion response vs HCV core determined by ELISPOT



Dr. Naglaa Shoukry

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Five *de novo* responders

Hepatic fibrosis stratification. Cluster analysis between responder and non-responder

Patients	Fibrosis score Pre-treatment	Fibrosis score Post-treatment	Responder (R) Non responder (NR)
4-m	1	0	R
9-m	2	0	R
12-f	1	0	R
7-m	1	0	R
13-f	1	0	R
10-m	0	0	R
6-m	2	3	NR
3-f	0	1	NR
1-f	0	1	NR
15-m	1	2	NR

Table: Hepatic fibrosis score of patients

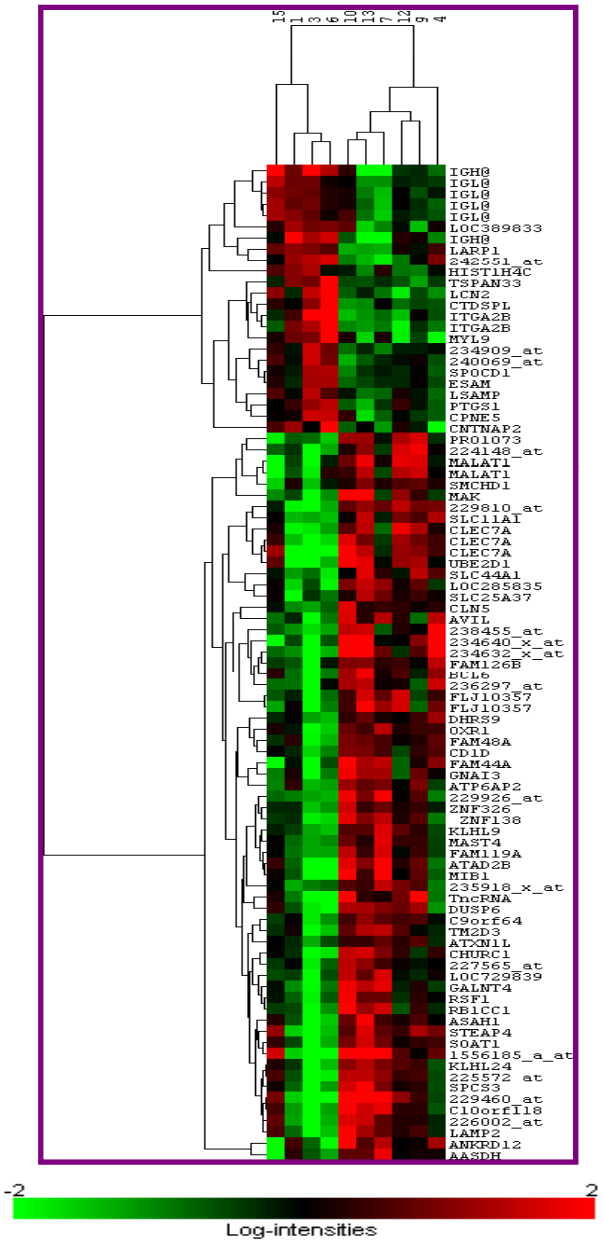

Figure: Expression Cluster realized with the 89 genes with significant differences between the class responder vs. non-responder patients. p≤0.01. (N=10).

Conclusions

- The VLPs:
  - elicited a potent IgG antibody response, with a mixed pattern of IgG subclass.
  - generate a strong lymphoproliferative response.
  - generate a high IFN-gamma secreting cell.
- The results suggest that the effective immunotherapy is correlated with the enhanced $CD8^+$ T-cell responses generated by vaccination
- VLPs can be exploited as platforms to increase the immunogenecity of poorly immunogenic antigens, including self proteins.
- All these features support the possible use of the VLPs expressed in *Pichia pastoris* and *E. coli* as a therapeutic and prophylactic vaccine candidates.

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