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Recombinant VLP based human vaccines for emerging markets

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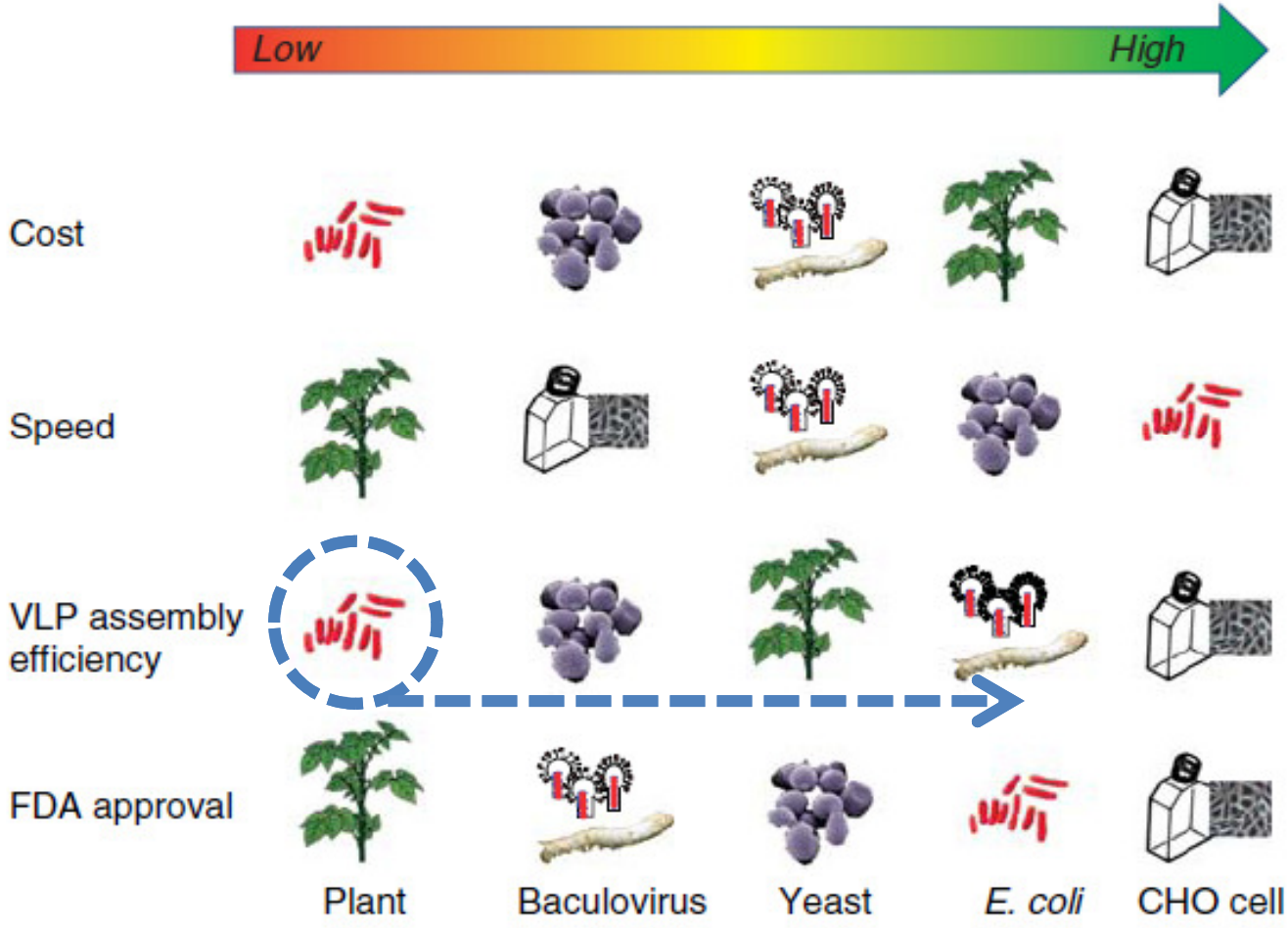
May 21, 2012 @ Albufeira, Portugal

Outlines

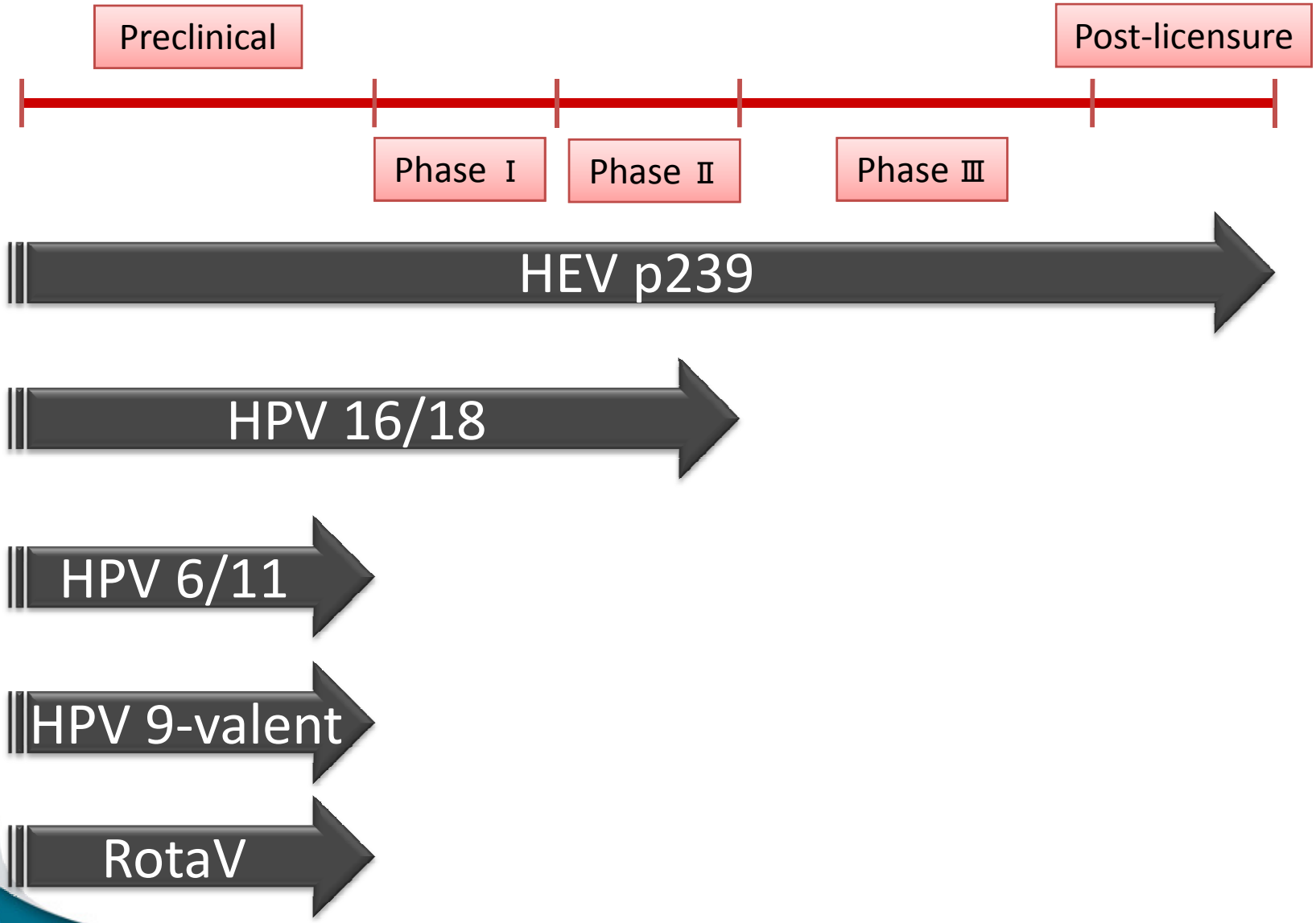
- Production platforms for recombinant VLP based vaccine
- Pipeline of VLP based vaccines
- First licensed HEV vaccine
- Other VLP based vaccines under development

Production platforms for recombinant VLP based vaccine



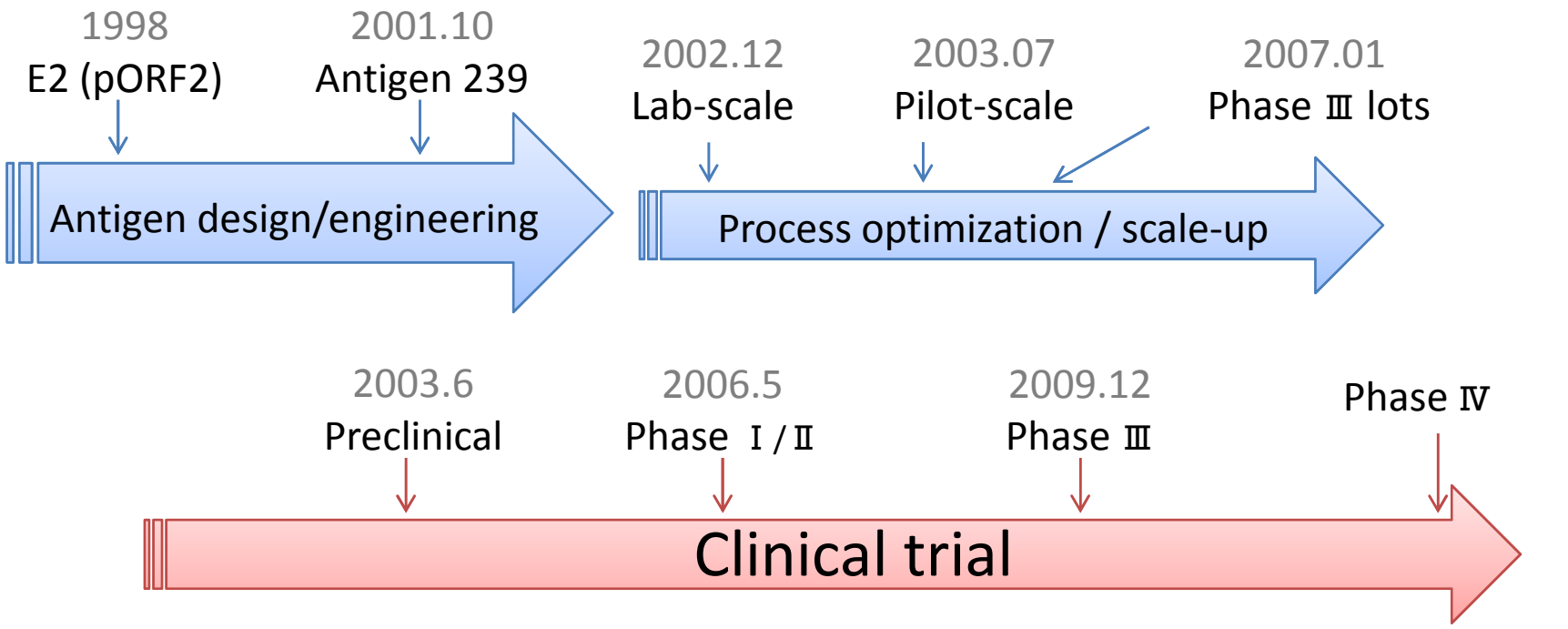
Cho, H. J., Y. K. Oh, et al. (2011). "Advances in human papilloma virus vaccines: a patent review." Expert Opin Ther Pat.

Pipeline of VLP based vaccines



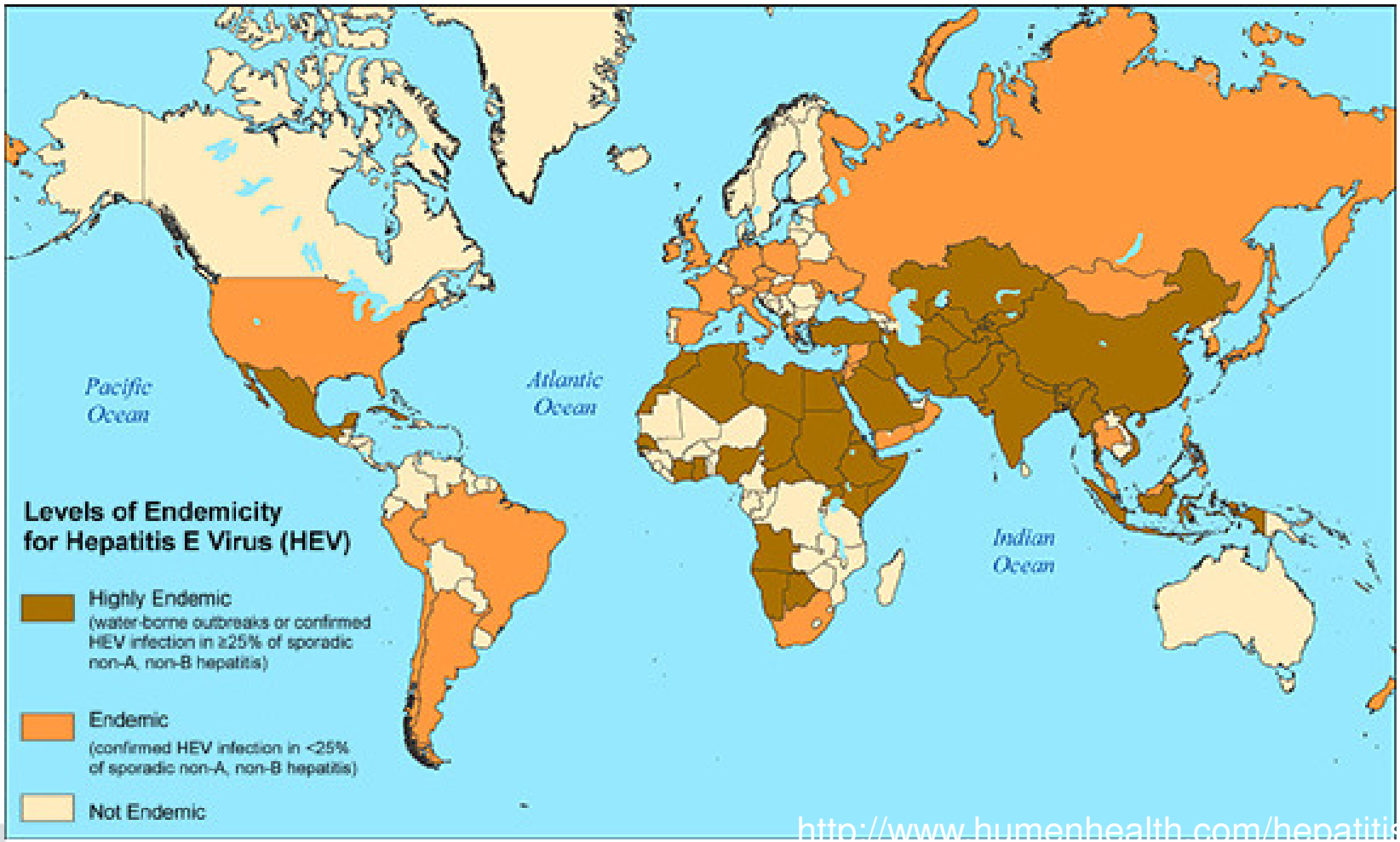
First licensed HEV vaccine

Milestones



Wu, et al. *Human Vaccine*, 2012

Geographic distribution of Hepatitis E

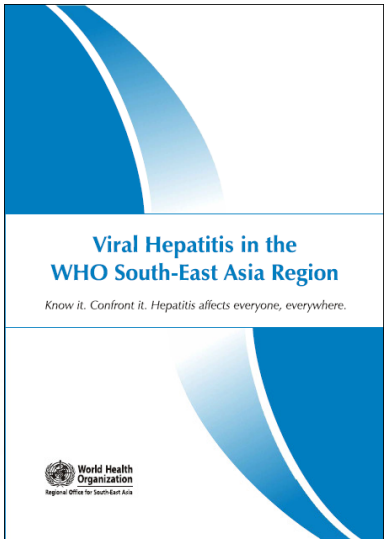


http://www.humenhealth.com/hepatiti

Global disease burden of Hepatitis E

Hepatitis E

WHO, 2011



- Hepatitis E infection is a viral liver disease that can cause mild to severe illness.
- It is spread by fecal-oral (or stool to mouth) route when a person ingests food or drink contaminated by an infected person's stool.
- The disease is closely associated with poor sanitation and a lack of personal hygiene habits, such as hand-washing.
- An estimated 14 million symptomatic cases of hepatitis E infection, with 300 000 deaths and 5200 stillborns occur annually in the world.
- Epidemics can show rapid growth and with high mortality among pregnant women.
- There is an evidence of food-borne transition of hepatitis E worldwide.
- Improved sanitation is the most effective way to combat the disease.
- No vaccine is commercially available for this infection.

R & D of HEV239 vaccine

1998 E2 (pORF2)

2001.10 Antigen 239

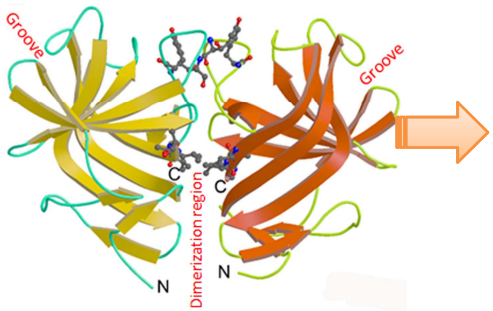
Antigen design/engineering

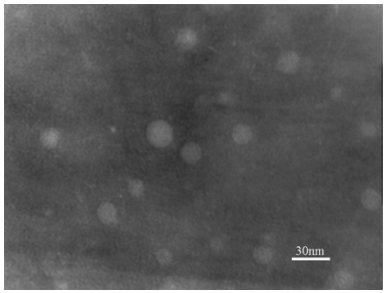
2002.12 Lab-scale

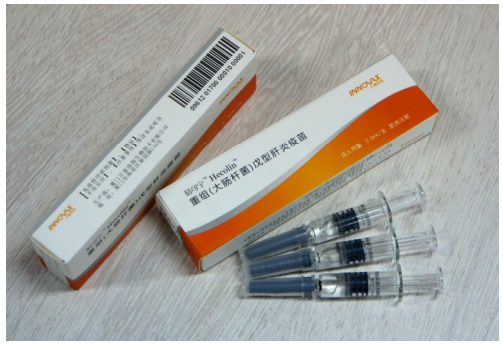
2003.07 Pilot-scale

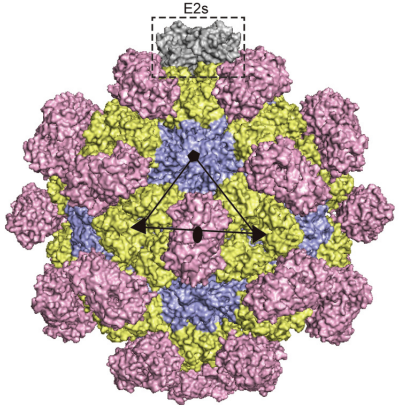
2007.01 Phase Ⅲ lots

Process optimization / scale-up





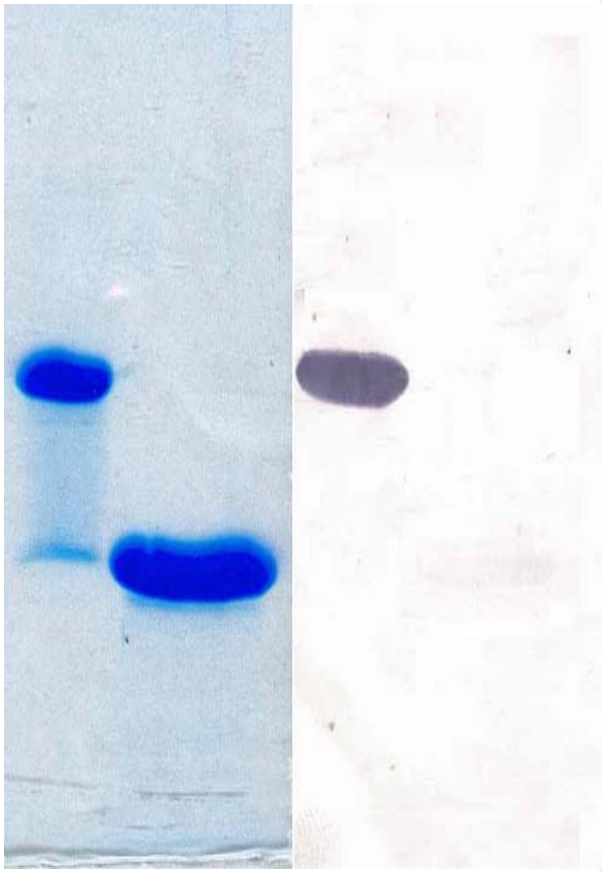




T=1 HEV VLP (PDB No.2ZTN)

Much preferred immuno-reactivity of patient sera to E2 (over monomeric form)

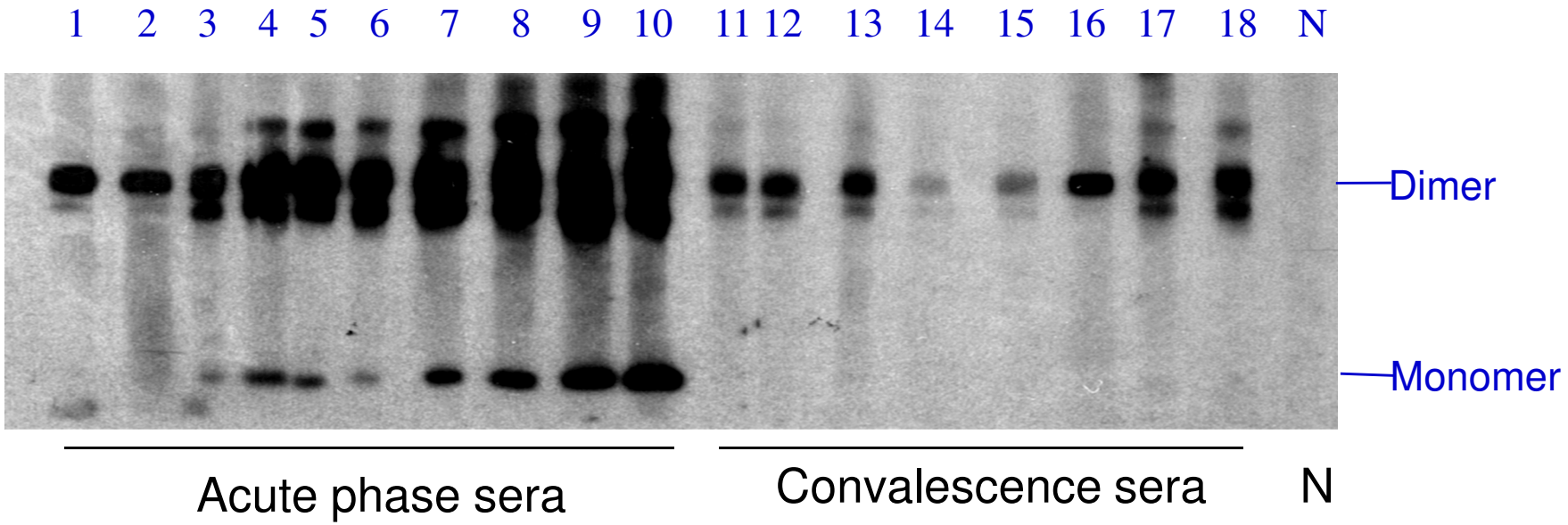
- pORF2 forms dimer, or higher order assemblies upon over expression
- Sucessful expression of HEV pORF2 fragment, E2, in *E. coli*
- Lost immuno-reactivity of E2 dimer upon denaturation into monomer



Western Blot

Zhang et al. *J Med Virol* 2001.

Preferred reactivity of patient sera to dimeric form (E2) of pORF2



- No reactivity to monomer antigen in convalescence sera
- Immunodominant epitopes of HEV pORF2 residing on dimeric form

Protection of rhesus monkeys from infection

(immunization with E2)

Table 4
Detection of HEV genome in stool and peripheral blood samples from monkeys after experimental infection with HEV[a]

Group	Monkey	Day after challenge									
		3	5	7	9	11	13	15	17	19	20
Test	M1	−	−	−	−	−	−	−	−	−	−
	M2	−	−	−	−	−	−	−	−	−	−
	M3	−	+	−	−	−	−	−	−	−	−
Control	M5	−	+	+	+	+	+*	+	+	−	−
	M7			|	|*	|	|	|	|		
	M8	−	−	+	+	+	+	+	+	−	−

[a] Stool specimens were collected every 2 days and peripheral blood were collected every week from the animals after virus challenge for 4 weeks The HEV genome was detected in the plasma samples by RT-PCR and, in the fecal and the PBMC samples by immune capture RT-PCR. '+ indicates a positive detection in the fecal samples and '−' indicates a negative finding. (*) indicates detection of the viral genome in the PBMC specimens.

Zhang et al. *J Med Virol* 2001.

Identification of two major neutralizing epitopes of HEV pORF2

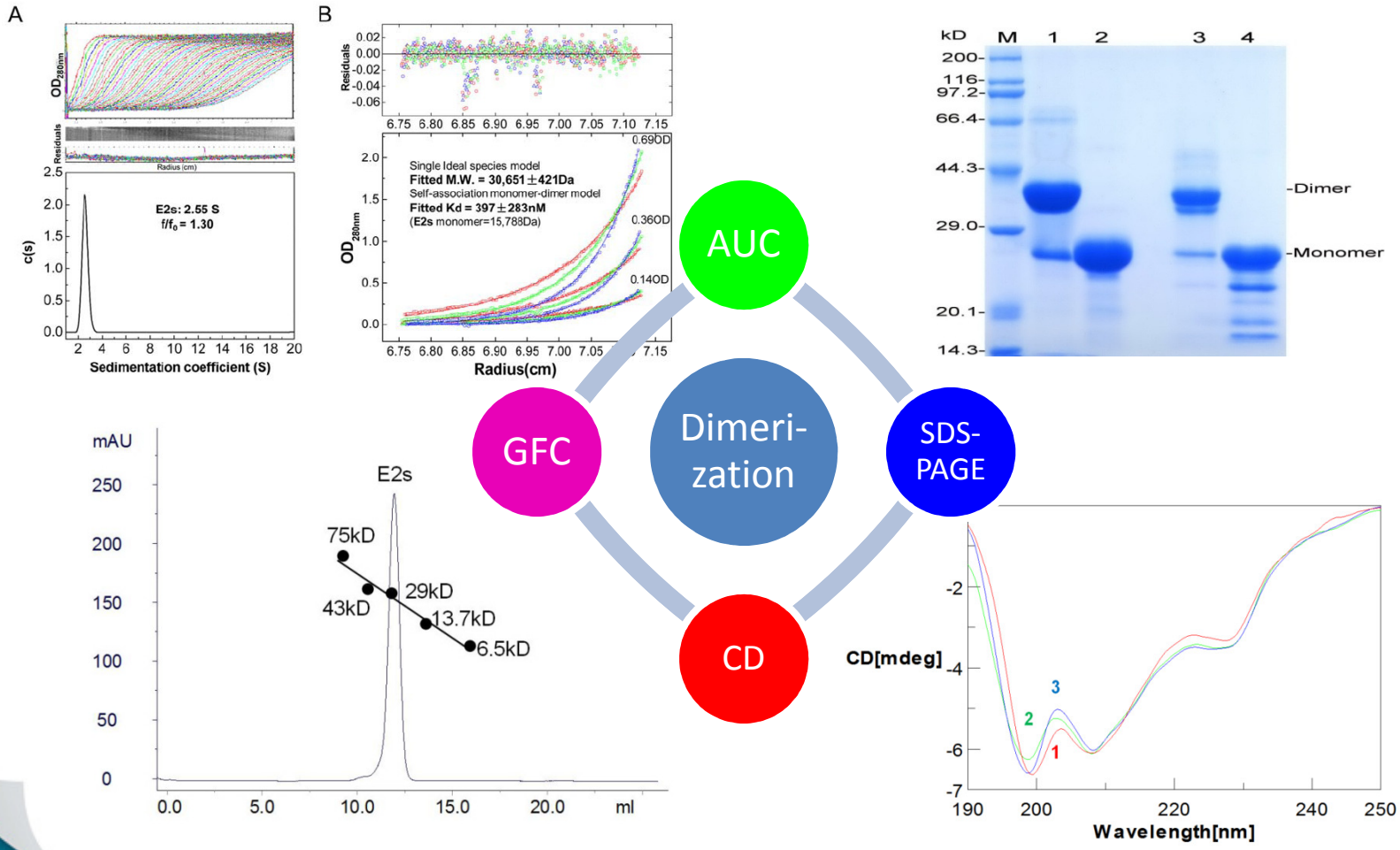
Table 2
Neutralization of HEV infectivity by E2 specific monoclonal antibodies in a rhesus monkey model

MAbs	Monkey	ALT (peak/pre-infection)	Onset of stool virus shedding (days p.i.)	Days of stool virus shedding	Anti-HEV IgG seroconversion (weeks p.i.)
Ctrl	KF25	3.3	8	47	5
	KF26	4.5	4	65	5
	KF27	3.6	16	25	5
8C11	KF16	1.2	19	22	5
	KF17	1.8	26	15	6
	KFI8	0.9	10	49	5
8H3	KFI9	1.0	16	36	7
	KF20	1.4	19	40	6
	KF21	1.1	16	83	6
8C11 and 8H3	KF22	2.3	23	18	5
	KF23	1.3	40	12	8
	KF24	0.8	Unconverted	0	Unconverted

A strain of genotype I HEV was mixed with 8H3 or 8C11 alone or in combination and incubated at 4 °C overnight and 37 °C for a further 2h and then used to inoculate rhesus monkeys. The inoculum originally contained 1000 virus genomic copies, which was estimated previously to be equivalent to about 100 infective unit for these animals. 8C11 contained in the mixtures had been diluted to a titer of $1:10^5$ and 8H3, to $1:10^4$. Serum and stool samples were taken before and twice weekly after infection for determination of serum levels of ALT and HEV antibody and for shedding of the HEV RNA in stool.

Zhang, et al. *Vaccine*, 2005

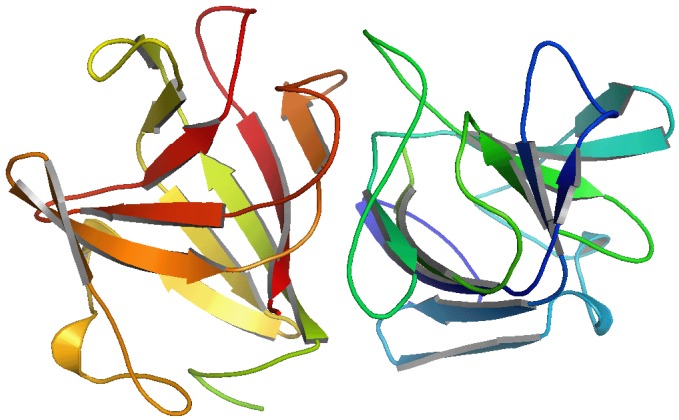
Dimerization of HEV pORF2
(E2s domain-149aa)

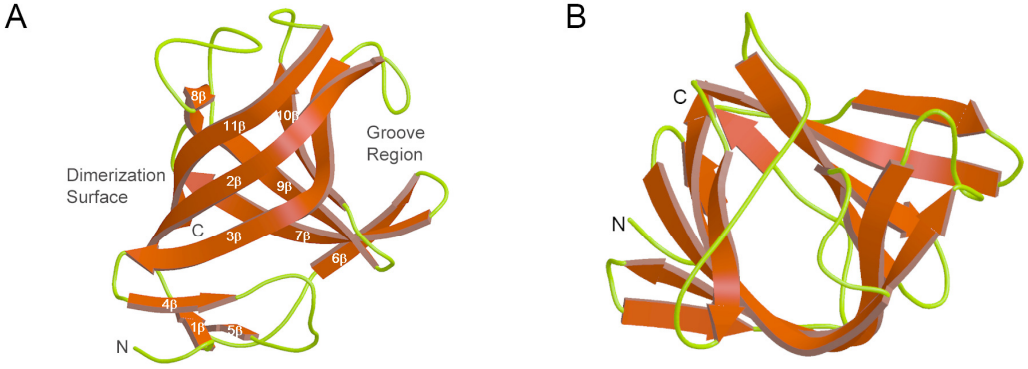


Li, et al. *PLoS Pathogen*, 2009.

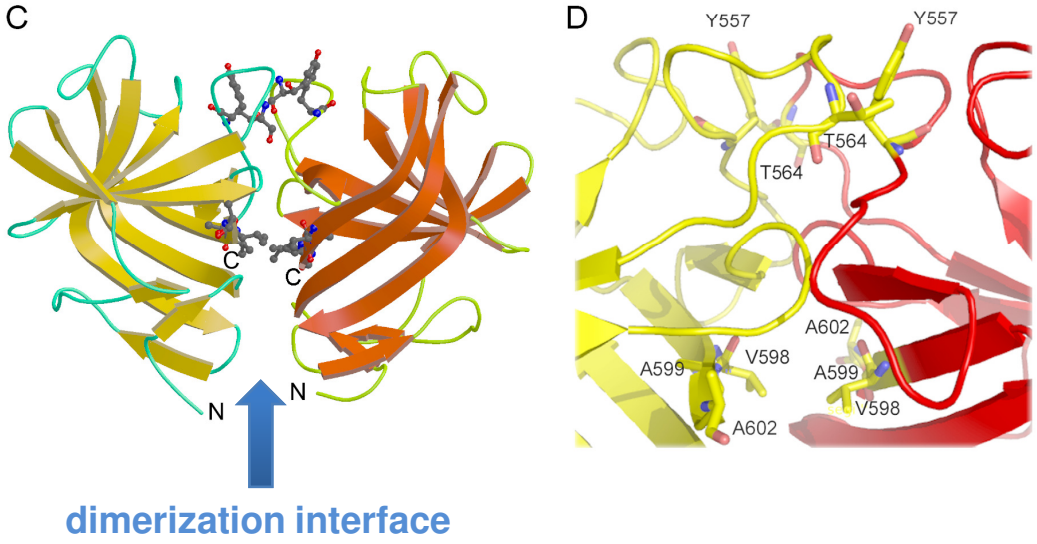
Structure of HEV pORF2

(E2s domain-149aa)





- Typical capsid protein folding: **β-barrel**
- Dimerization: strong **hydrophobic interactions**
- Groove region: **flexible loops**

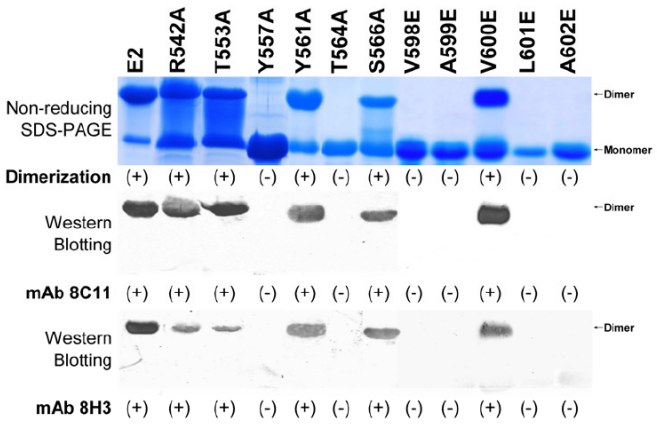


dimerization interface

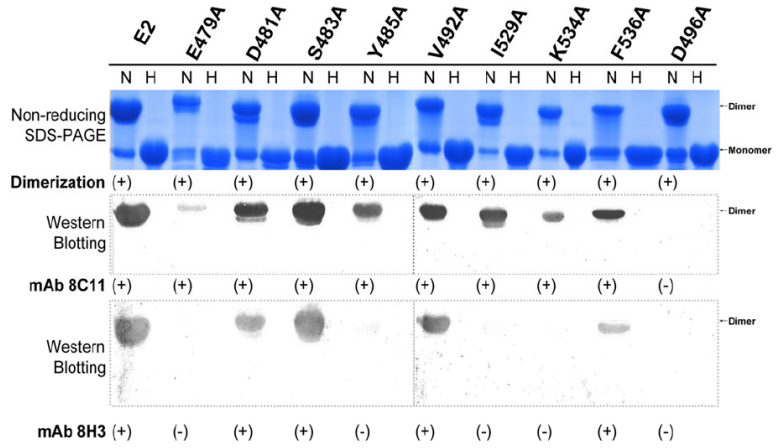
Li et al. *PLoS Pathogens*. 2009

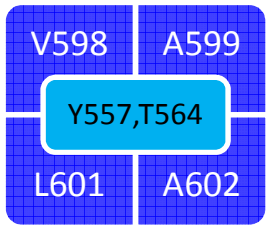
Mapping of key residues on E2s

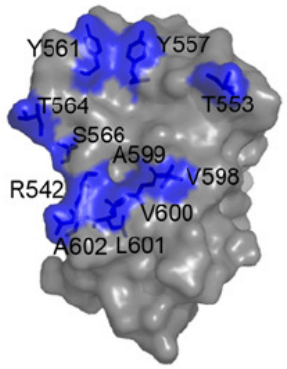
✓ Dimer interface

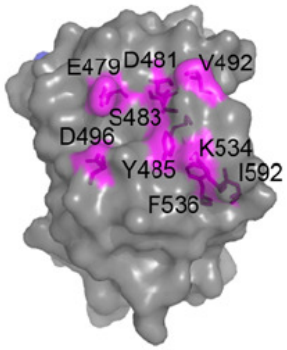


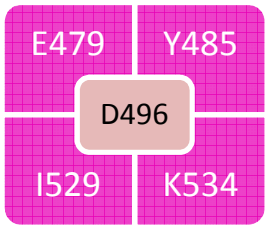
✓Immune-predominant neutralizing sites





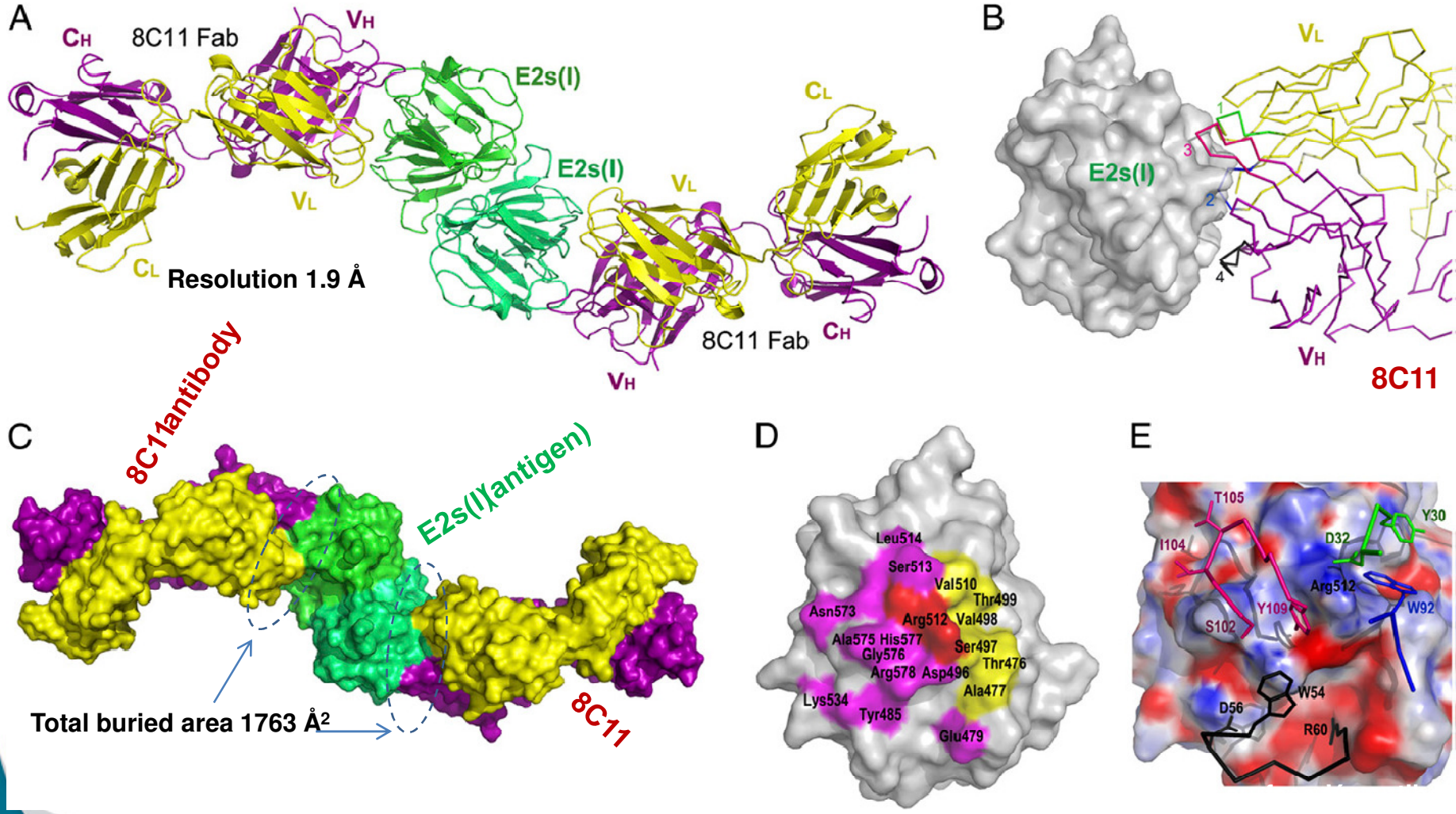






Li, et al. *PLoS Pathogen*, 2009.

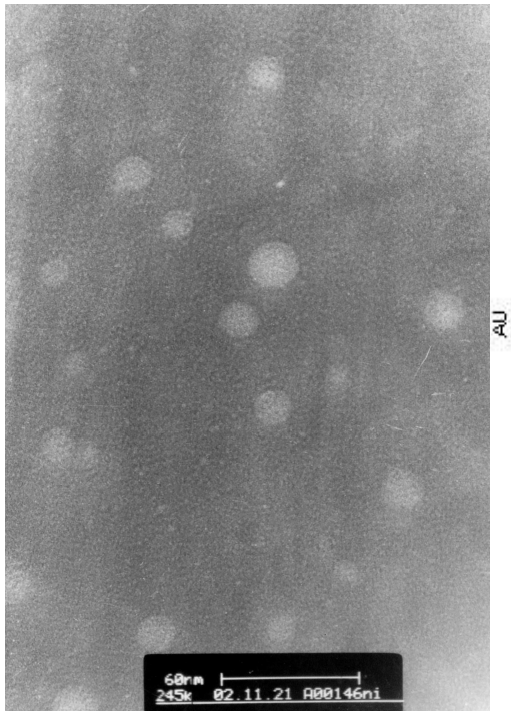
Structure of E2s:8C11 mAb complex



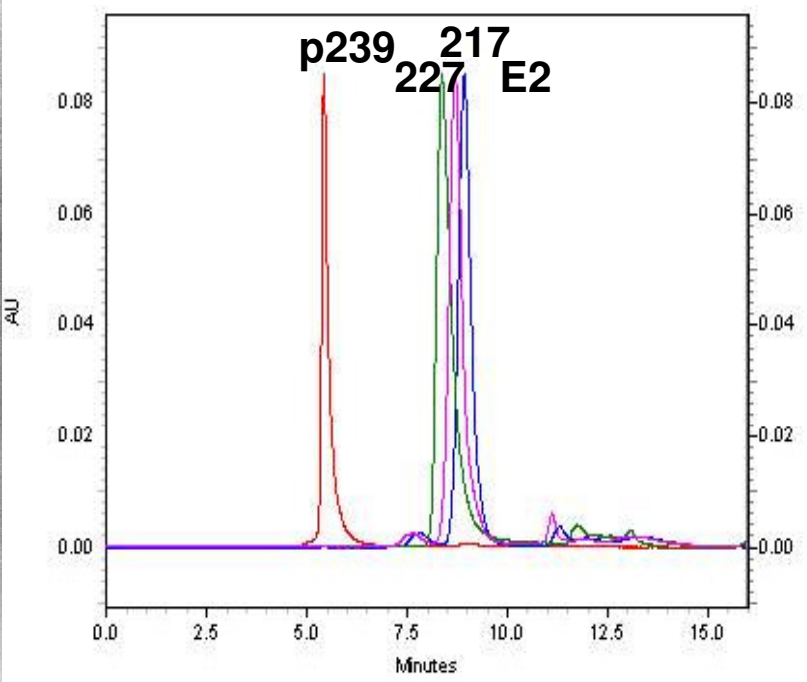
8C11 epitope on E2s: Asp496-Thr499, Val510-Leu514 and Asn573-Arg578

Tang *et al., PNAS.* 2011.

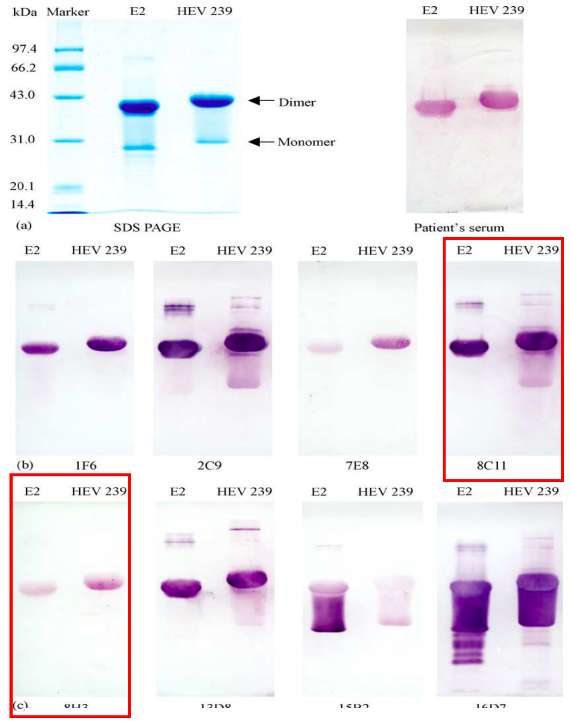
Enhancing immunogenicity by making multimeric VLP antigen



Electron microscope



Gel filtration by HPLC
(GSW3000xl column)



SDS-PAGE and WB
of p239 vs. E2

- Successive N terminal extension of E2 generated particulate HEV 239, retaining dimerization capability and major neutralization epitopes.

Li et al. *JBC, Vaccine.* 2005

Enhanced Immunogenicity of 239

Table 1
Antibody response of mice to the HEV 239 and E2 vaccines

HEV 239 vaccine		E2 vaccine	
Dose (μg)	Seroconvertion no./ inoculated no.	Dose (μg)	Seroconvertion no./ inoculated no.
20	8/8	5	0/4
6.67	8/8	10	0/4
2.22	8/8	15	0/8
0.74	8/8	20	0/4
0.25	7/8	30	1/8
0.08	3/8	60	2/8

Balb/c mice were inoculated once with the indicated doses of HEV 239 or E2 and bled 4 weeks later for the determination of HEV antibodies. Both vaccines were suspended in alum adjuvant.

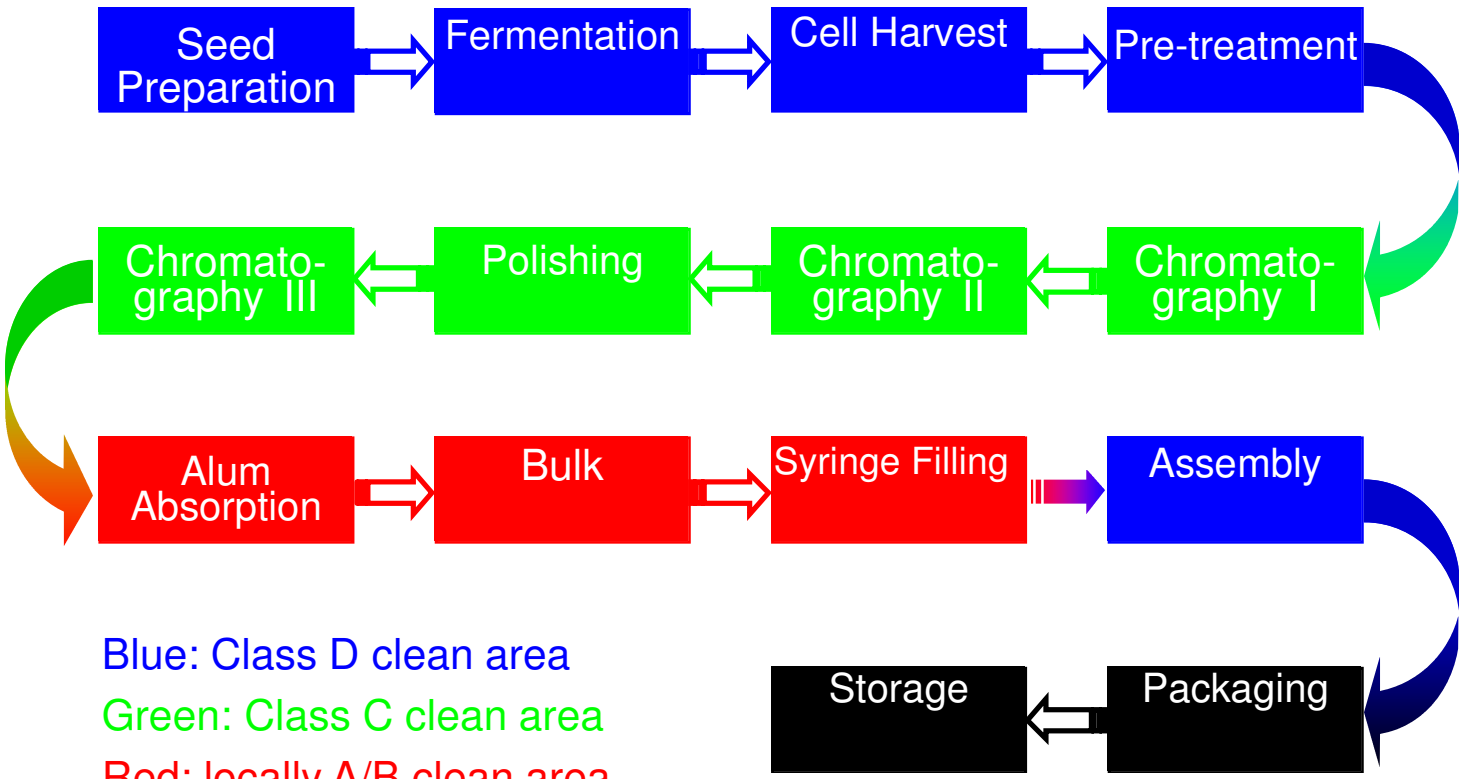
Li et al. Vaccine 2005.

HEV 239 vaccine efficacy in rhesus monkeys

Virus dose (MID50)	Group	Pre-infection Ab (IU)	Infection	Hepatitis	Efficacy against infection (CI)	Efficacy against hepatitis (CI)
10^5	Vaccine	1,168	3/12	0/12	75% (46.2%-90.9%)	100% (75.3%-99.8%)
	Control	<2	6/6	5/6		
10^2	Vaccine	1,599	0/12	0/12	100% (75.3%-99.8%)	100% (75.3%-99.8%)
	Control	<2	12/12	1/6		

Li et al. Vaccine 2005.

Process flowchart of HEV 239 vaccine



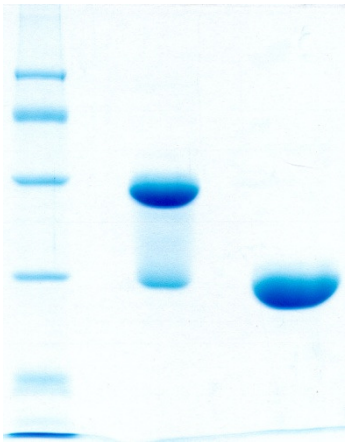
Blue: Class D clean area

Green: Class C clean area

Red: locally A/B clean area

Black: controlled area

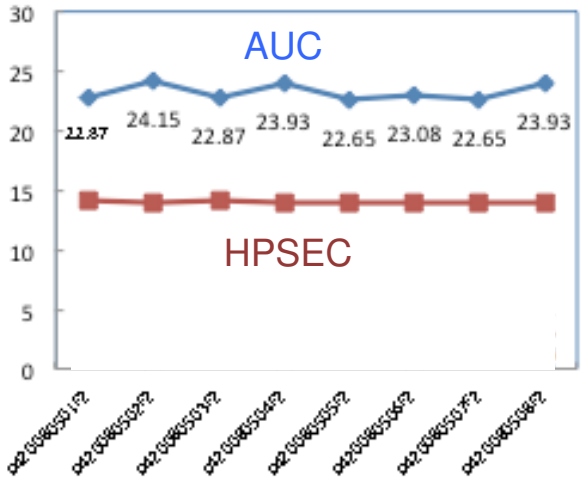
QC of p239 vaccine

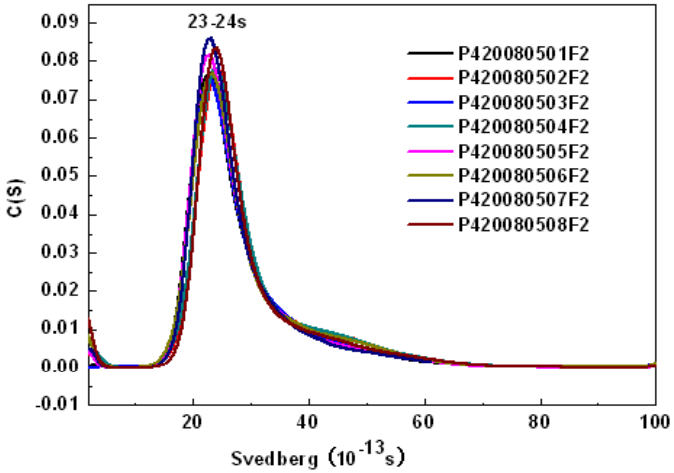


Product : HEV vaccine	Mfg. Date: Apr 2003
Batch No: 20030401	Pack size: 0.5 ML

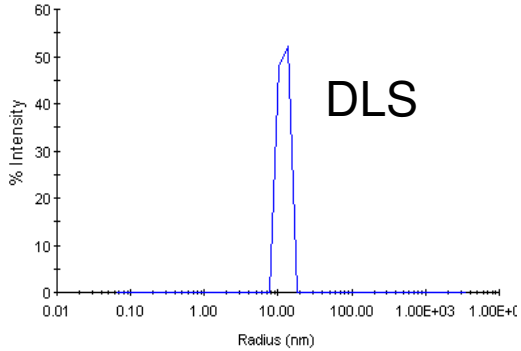
S.No	Tests	Specifications	Result
1	**Fill volume**	**0.5 – 0.6 ml**	Passes
2	**Appearance**	**White turbid liquid**	Whitish turbid liquid
3	**Identity–ELISA**	**Should identify**	Identifies
4	**Al^{+++} content**	**$Al(OH)_3$ 1.4~1.8 mg / ml**	0.56 mg / ml
5	**Thiomersal content**	**39.0 – 67.0 µg / ml**	50 µg / ml
6	**pH**	**6.1-7.4**	6.65
7	**Sterility**	**Shall comply**	Passes
8	**Abnormal toxicity**	**Shall comply**	Passes
9	**Bacterial endotoxins**	**Less than 10 EU / 0.5ml**	Passes
10	**Relative potency (ED50)**	**Less than 1.5 µg**	0.113

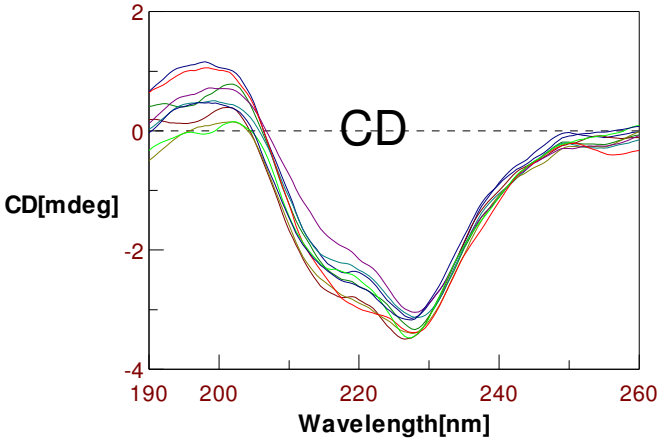
Characterization of p239 particles

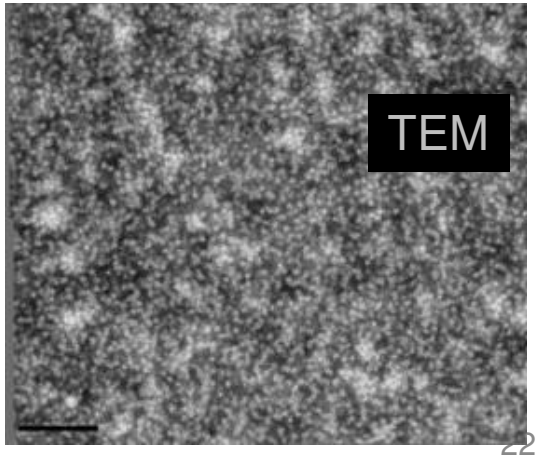




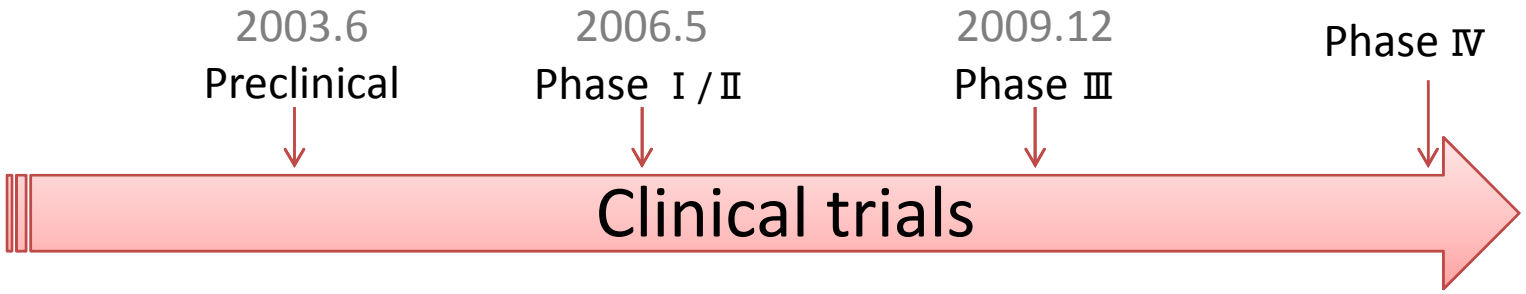
AUC
-SV







Clinical trials of HEV vaccine



Guangxi, China

Jiangsu, China

Clinical trials of HEV vaccine

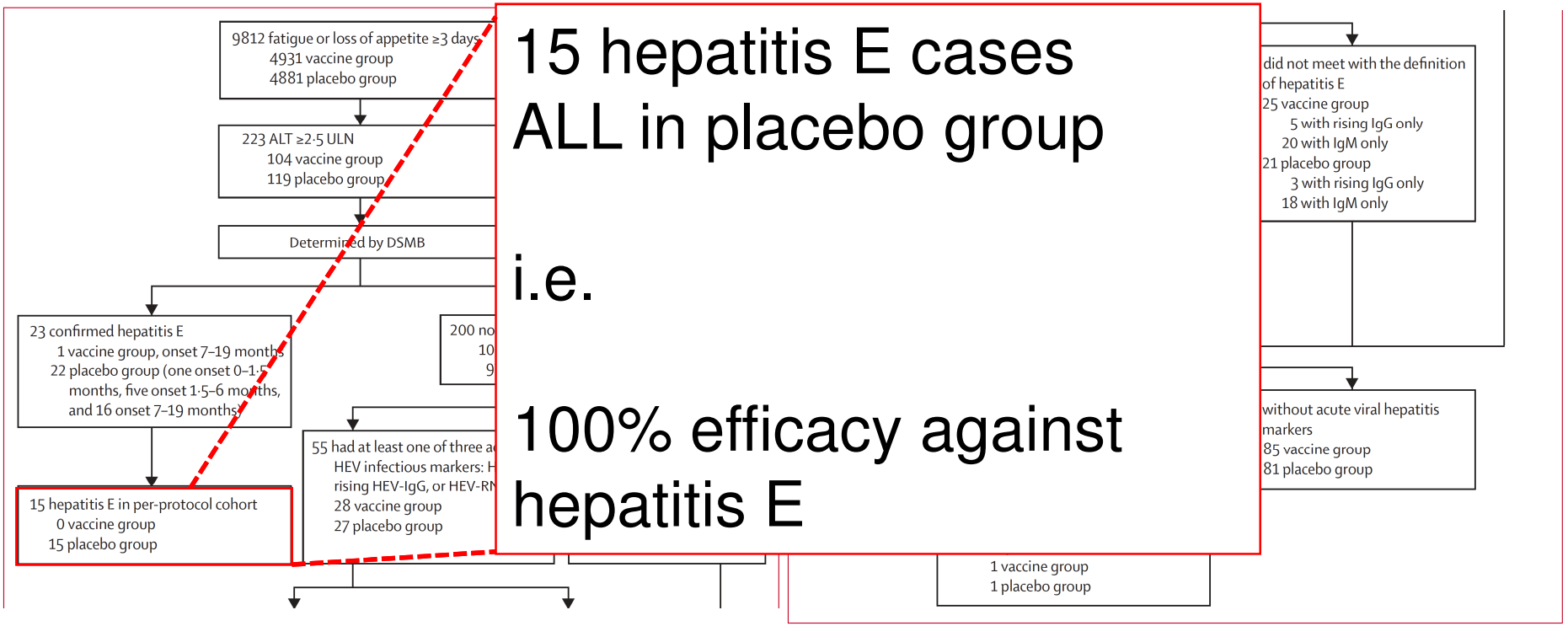
Table 1. Clinical trials of the recombinant HE vaccine during late-stage development of Hecolin®

Trial	Study Purpose	No. of patients	Key conclusion	Ref.
Phase Ia (2005.1–2005.3)	Safety	44	✓ Well tolerated*	
Phase IIa (2005.4–2005.11)	Safety, Dose schedule	457	✓ Well tolerated* ✓ 3 doses with a regimen of 0–1-6 min was selected	
Phase IIb (2005.4–2005.11)	Safety, Dosage escalation	155	✓ Well tolerated* ✓ Seroconversion rate was 100% in all the subjects ✓ 30-μg dose was selected for a Phase III clinical trial	
Phase III (2007.8–2009.5)	Safety, Efficacy, Immunogenicity	112 604	✓ Well tolerated in the general population* and showed preliminary evidence for safety in pregnant women.*& ✓ Efficacy after three doses was 100% (95% CI 72·1–100). ✓ 98.7% of subjects had anti-HEV IgG response after receiving HE vaccine	

Notes: *No vaccine-related SAEs were reported. &68 pregnant women were inadvertently vaccinated with 1 or more doses of HE vaccine or placebo vaccine during Phase III clinical trial; the AEs occurred in this population were analyzed.

Wu, et al. *Human Vaccine*. 2012

Phase III Clinical trials of HEV vaccine



15 hepatitis E cases
ALL in placebo group

i.e.

100% efficacy against hepatitis E

Flowchart of surveillance and certification of acute hepaitis E in phase III

Zhu, et al. *Lancet*. 2010

Publications

1. Wu, et al. *Hepatology* 2011(in press)
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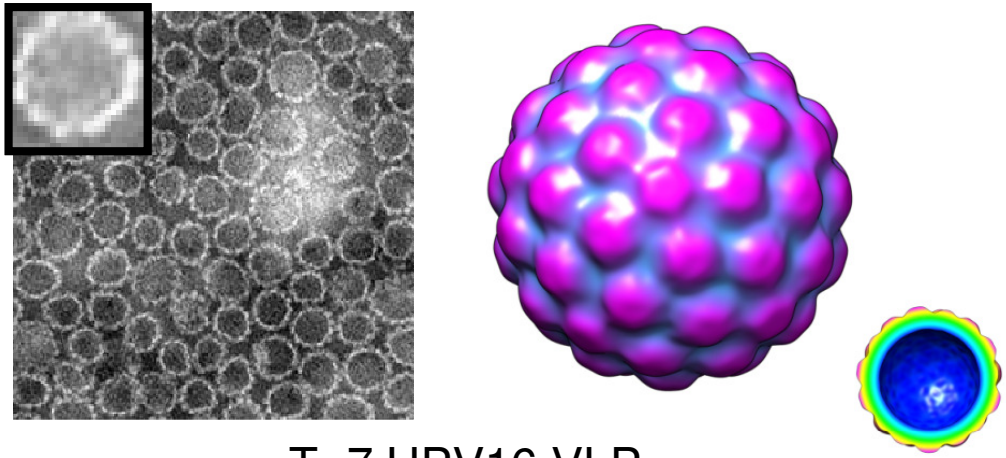
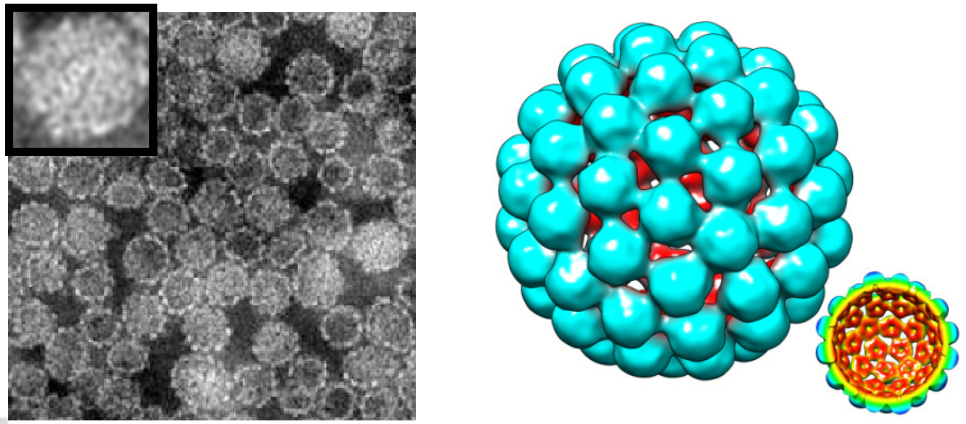
Other VLP based vaccines under development

- HPV16/18 bivalent vaccine
- HPV6/11 bivalent vaccine
- HPV16/18/52/58/31/33/45/35/59 9-valent vaccine
- RV quadrivalent vaccine

HPV16/18 bivalent vaccine



T=7 HPV16 VLP

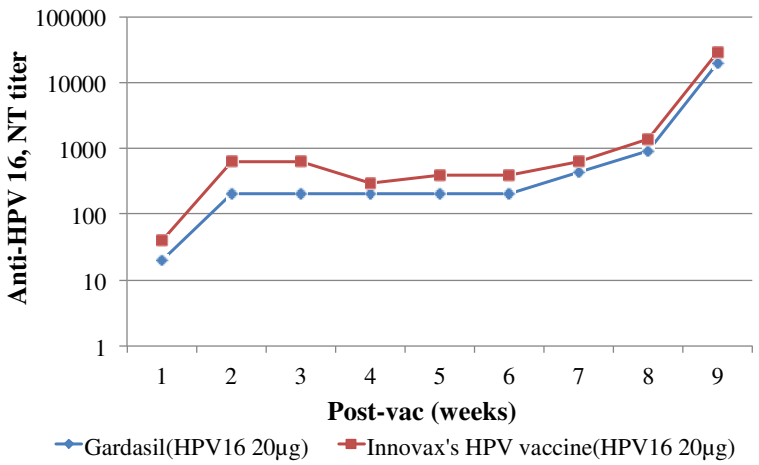


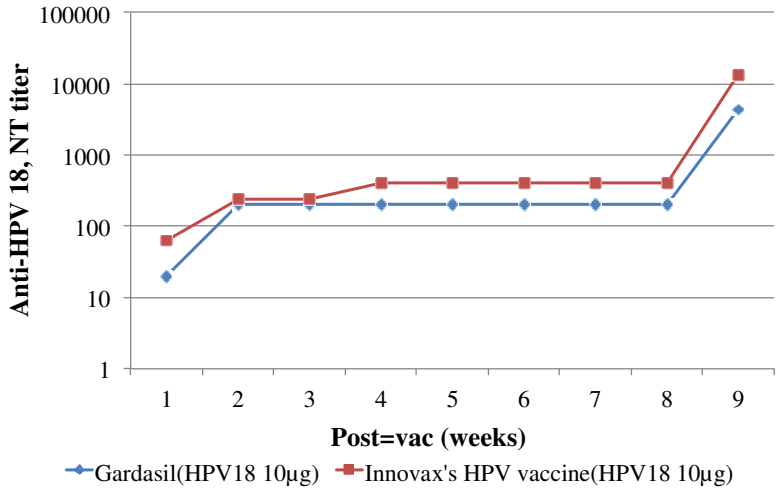
T=7 HPV18 VLP

HPV16/18 bivalent vaccine

- *E. coli* expression system
- HPV L1 virus-like particles

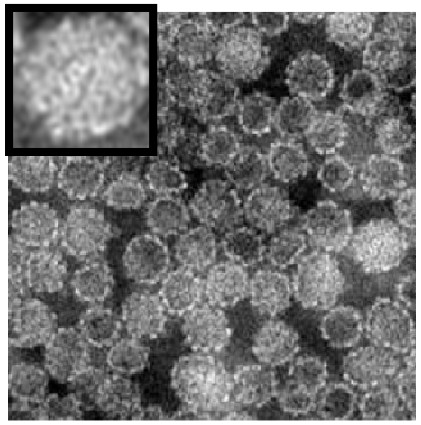
Immunogenicity of HPV16/18 bivalent vaccine in rehsus monkeys

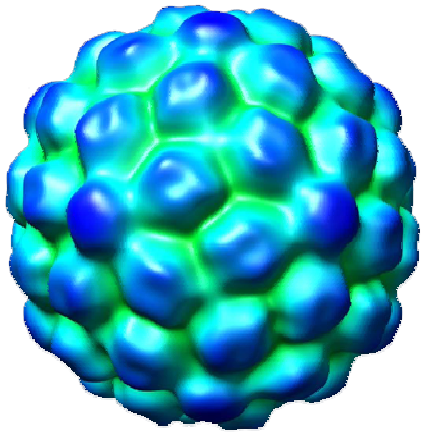




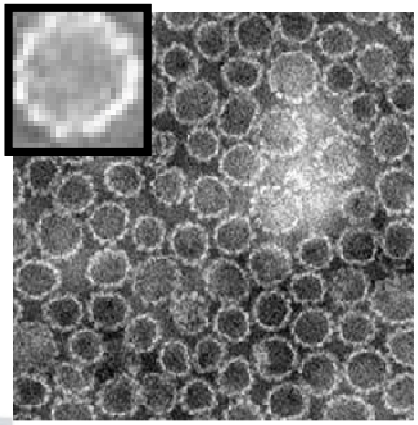
✧ Comparable neutralizing antibody levels

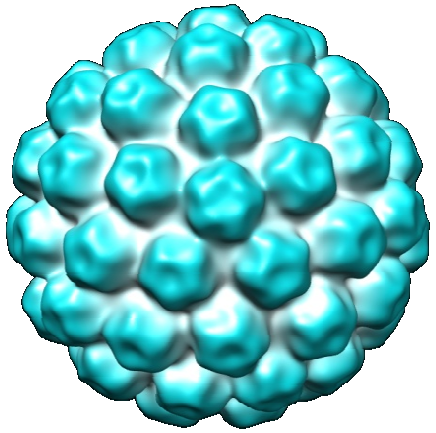
HPV6/11 bivalent vaccine





T=7 HPV6 VLP



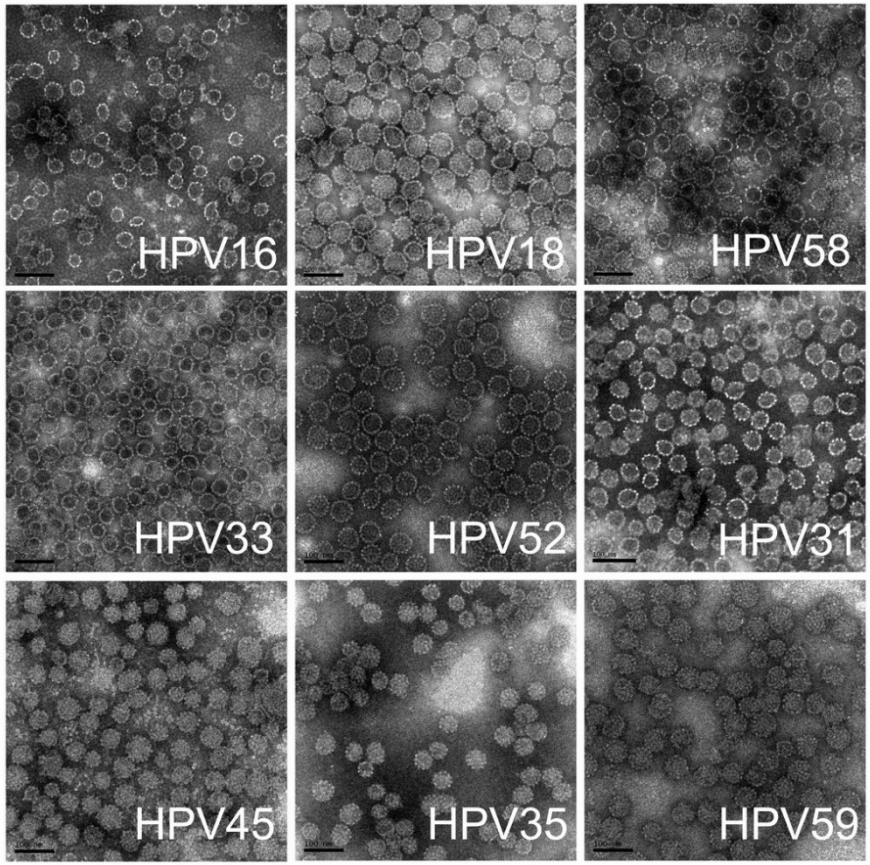


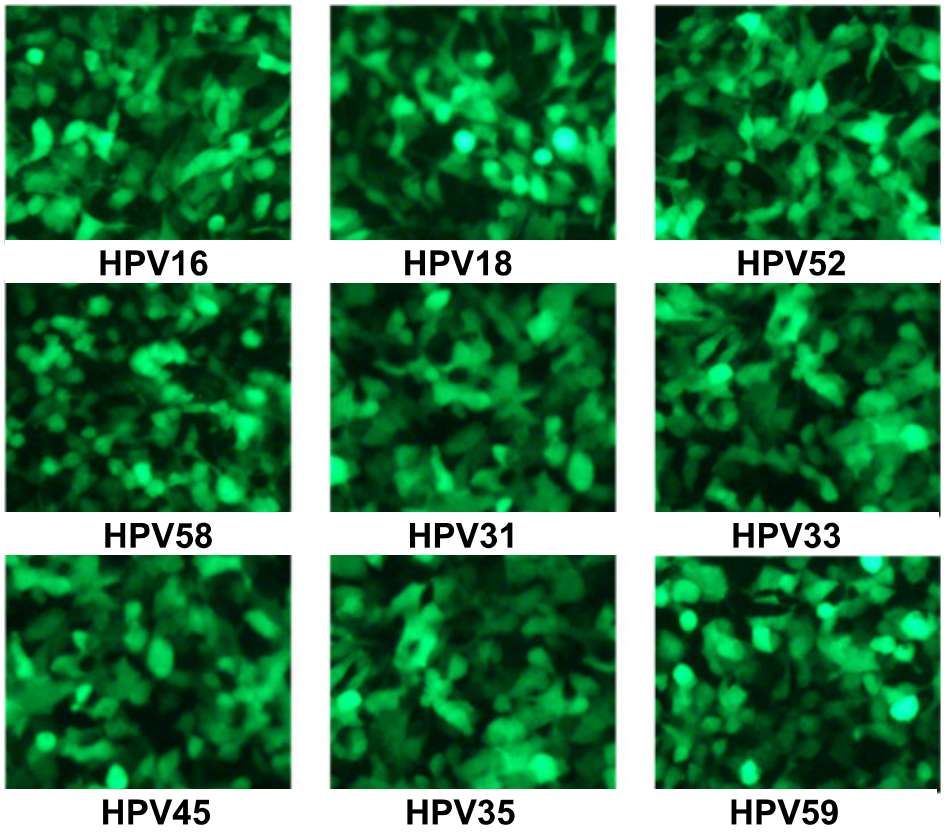
T=7 HPV11 VLP

HPV6/11 bivalent vaccine

- *E. coli* expression system
- HPV L1 virus-like particles

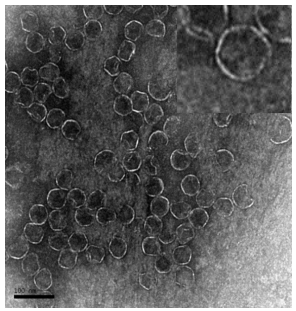
Ongoing efforts on VLP based vaccine



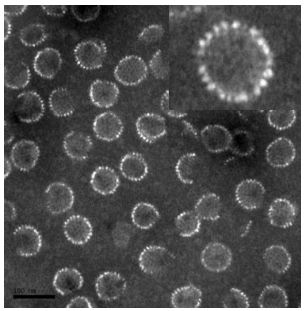


- High-risk type: HPV16/18/52/58/33/31/45/35/59 VLP
- corresponding pseudovirion-based neutralization assay

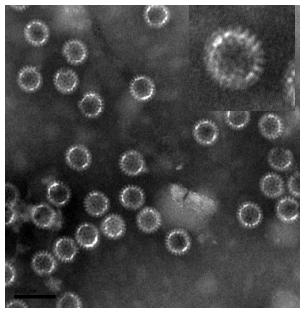
Ongoing efforts on VLP based nano-scale bioparticles



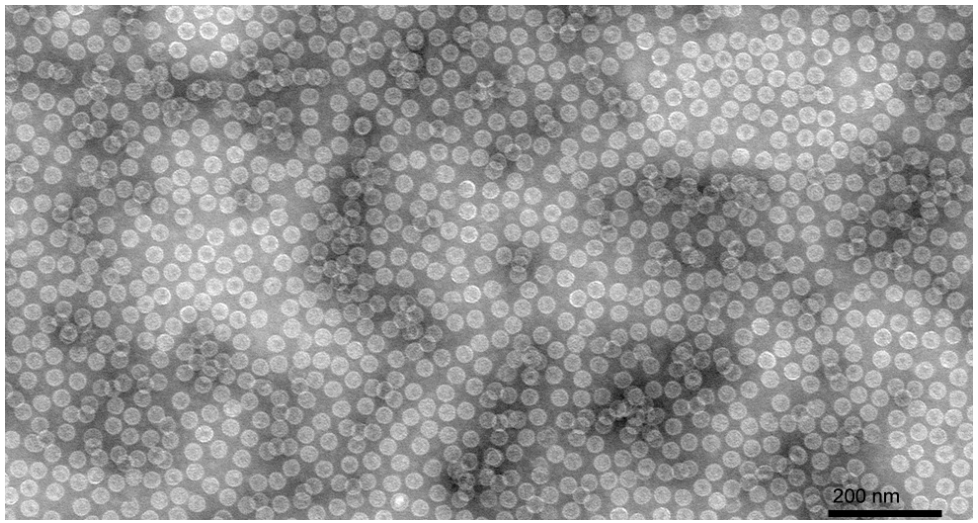
VP2 CLP



VP6 VLP



VP2/6 DLP



HBcAg particle

- Rotavirus VLP for vaccine
- HBcAg for epitope presentation
- HBcAg used in HB diagnostics

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Jun Zhang	J Sivaraman	Norm Olson	Yongzhong Gao
Ningshao Xia	Choyleong Hew	Timothy S. Baker	

Our team - NIDVD

Thank you!

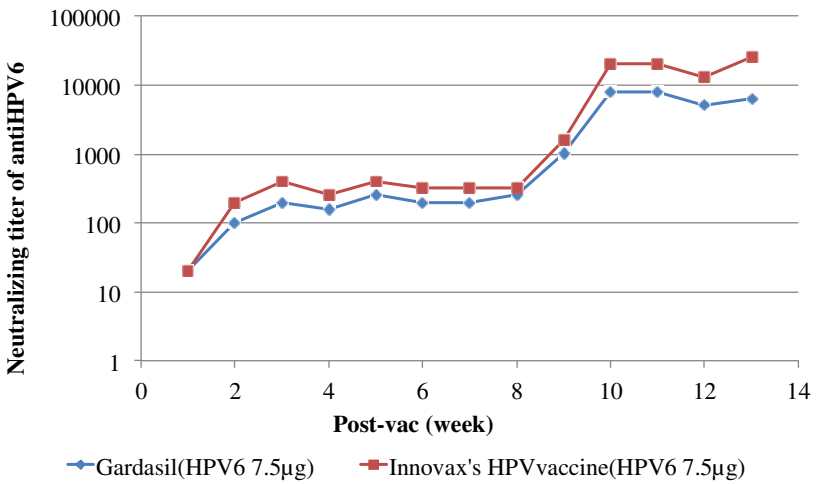
Welcome to Xiamen and Xiamen University!

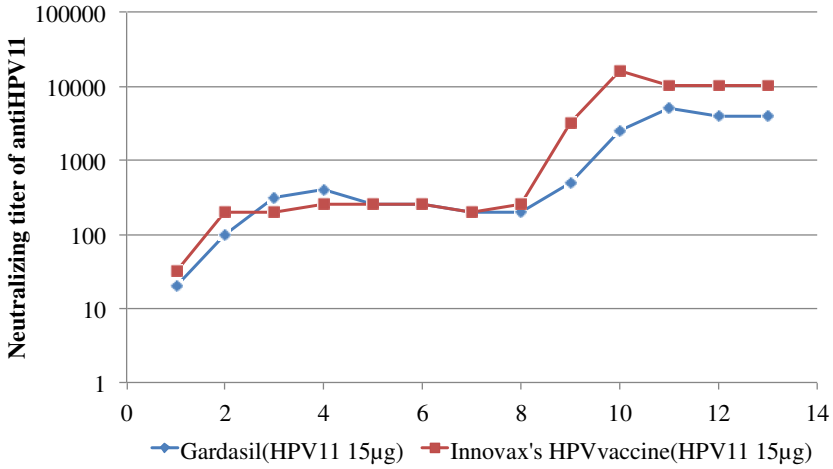



Thank you! Welcome to Xiamen !

Welcome to Xiamen University!

Immunogenicity of HPV6/11 bivalent vaccine in rehsus monkeys





✧ Comparable neutralizing antibody levels