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## **Prototype covalent HIV vaccine for inducing antibodies that neutralize genetically divergent virus strains**

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**Background:** The conserved CD4 binding site (CD4BS) of HIV is vulnerable to host immunity as it is essential for virus propagation. However, HIV infection and previous vaccine candidates do not induce neutralizing anti-CD4BS antibodies, presumably because the superantigen character of the CD4BS downregulates B cell adaptive immunity. Rare monoclonal antibodies to the exposed CD4BS residues 421-433 were detected by immunization with gp120 containing electrophiles that bind covalently to B lymphocytes (JBC 2009, 284:30627). This suggested covalent immunization as a strategy to overcome the poor CD4BS immunogenicity.

**Methods:** BALB/cJ mice and New Zealand White rabbits were immunized with synthetic E-416-433, a mimetic of CD4BS residues 421-433 containing electrophilic phosphonates at Lys residues. HIV neutralization was assayed using human peripheral blood mononuclear cells and primary virus isolates. Antibody binding was measured by ELISA. IgG was purified using immobilized protein G. Epitope-specific antibodies were purified using immobilized E-416-433.

**Results:** E-416-433 displayed specific binding to soluble CD4, suggesting that it adopts the native CD4BS conformation found on HIV surface. Immunization of mice and rabbits with KLH-conjugated E-416-433 induced polyclonal IgG and IgA responses with E-416-433 binding activity in blood and vaginal lavage fluid. Apparent affinity constants for antibody binding to E-416-433 were 36-41 nM. The sera and purified antibody fractions neutralized genetically diverse HIV strains from subtypes A, B, C and D. Epitope-specific antibodies purified using immobilized E-416-433 displayed enriched neutralizing and E-416-433 binding activities. Soluble CD4 inhibited antibody binding to E-416-433, confirming CD4BS specificity.

**Conclusions:** E-416-433 is the first HIV vaccine candidate capable of inducing polyclonal anti-CD4BS antibodies that neutralizes diverse HIV strains in experimental animals. Properties underlying success of the E-416-433 immunogen are mimicry of the native CD4BS conformation and ability to bind B cells covalently, a highly energetic reaction that increases CD4BS immunogenicity.

# Prototype covalent HIV vaccine for inducing antibodies that neutralize genetically divergent virus strains

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## *Disclosures*

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Covalent Immunology Products Inc: Planque and Nishiyama are consultants; Paul is Board Member

## Central Problems in HIV Vaccination

- No or marginal protection against infection by previous test vaccines
- Mutability of immunodominant viral epitopes
- Poor adaptive response to conserved epitopes essential for viral life cycle

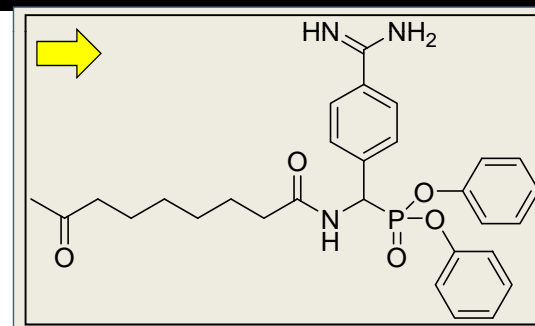
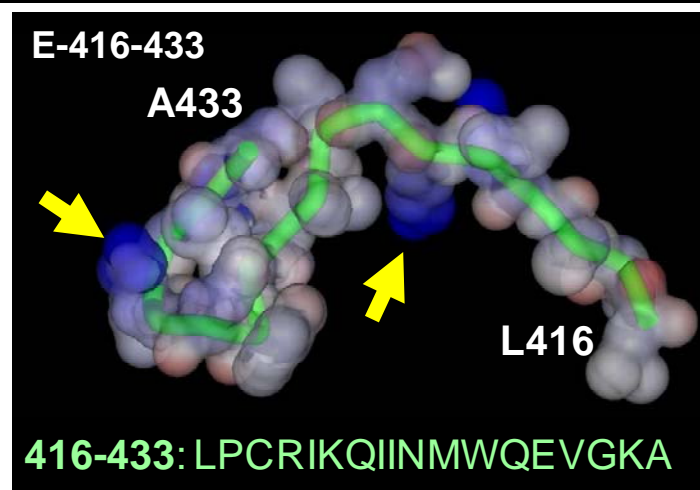
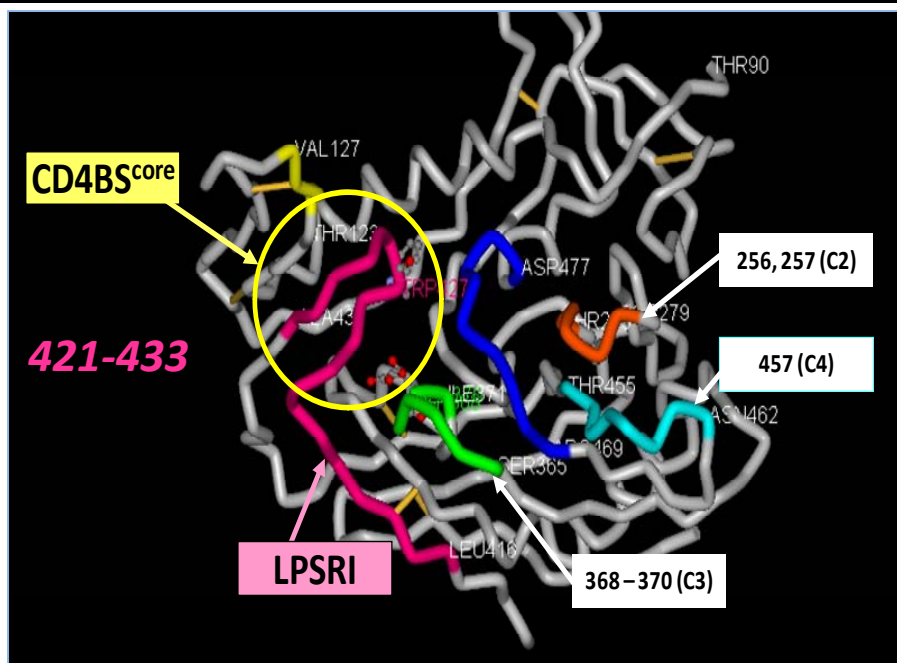
## Hypothesis

**Focusing the immune response at a structurally conserved epitope is necessary for effective HIV vaccination**

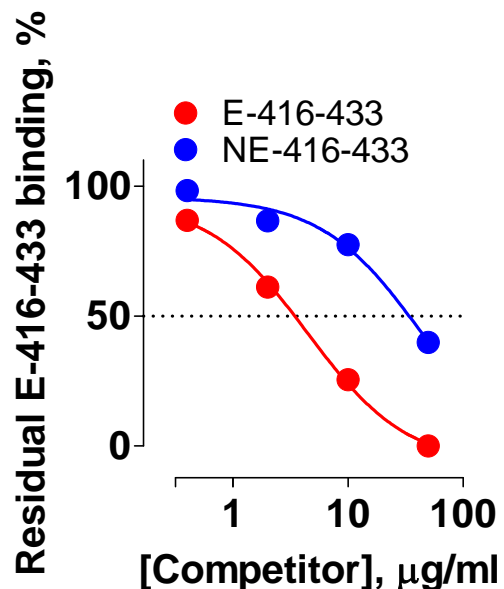
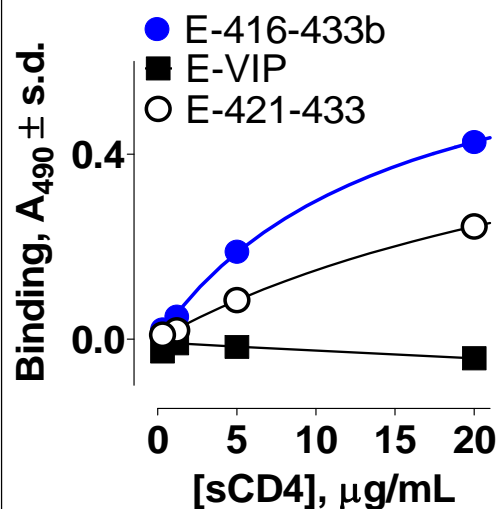
# Very few broadly gp120 neutralizing epitopes

<i>Abs</i>	<i>Epitope location<sup>a</sup></i>	<i>Function</i>	<i>Vaccine Relevance</i>		
			<i>Epitope accessibility</i>	<i>Ab neutralizing properties</i>	<i>Immunogenicity</i>
IgG b12, IgG VRC01	Conformational CD4BS epitope in exposed gp120 outer domain	Initial CD4 contact site	No evidence for limiting epitope exposure	Comparatively broad neutralization of diverse strains	Abs are rare. No immunogen available to induce Abs
IgG YZ23, scFv JL413, scFv JL427, IgA LTS	Linear CD4BS <sup>core</sup> epitope, residues 421-433; exposed bridging sheet	Peptide mimetics bind CD4	No evidence for limiting epitope exposure	Broad neutralization of diverse native HIV strains. No pseudovirion neutralization	Hypoimmunogenic. Long-term survivors produce potent neutralizing Abs.
IgG 2G12	Glycan substituents, e.g., at residues 332, 392	Not known	No evidence for limiting epitope exposure	Neutralizes some but not all strains	Abs are rare. No immunogen available to raise Abs
IgG X5, IgG 17b, IgG 48d	CD4-induced epitope; $\beta$ 2, $\beta$ 3, $\beta$ 20- $\beta$ 21 regions	Coreceptor binding	Exposure requires prior CD4 binding	Neutralize some but not all strains	Abs can be induced by infection/immunization
IgG 447-52D	V3 domain apex, residues GPXR	Coreceptor binding	Full exposure may requires CD4 binding	Limited breadth of neutralization.	Focusing Ab response to small peptide region may be difficult
IgG PG9, IgG PG16	Conformational epitope; V2, V3, C4 domain residues	Not known	No evidence for limiting epitope exposure	Broad pseudovirion neutralization. Native HIV tests awaited	Abs are rare. No immunogen available to induce Abs
Polyclonal Abs to CD4-independent gp140	Epitope not identified	Not known	CD4-independent but not CD4-dependent epitope may be exposed	Broad pseudovirion neutralization. Native HIV test awaited	Only CD4-independent envelope is immunogenic

# The vulnerable but challenging CD4BS<sup>core</sup>: E-416-433 mimetic

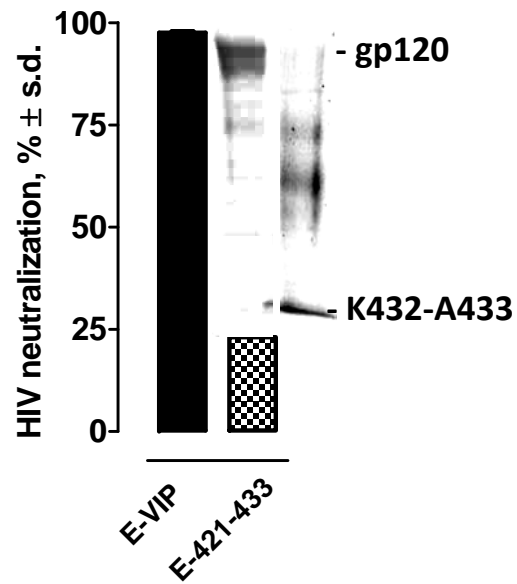


## sCD4 binding



***Solving conformational flexibility problems: E-416-433 binds CD4 120-fold better than previous CD4BS<sup>core</sup> peptides (K<sub>d</sub>=110 nM). E-416-433 is combined probe for reversibly-binding and catalytic CD4BS<sup>core</sup> reagents.***

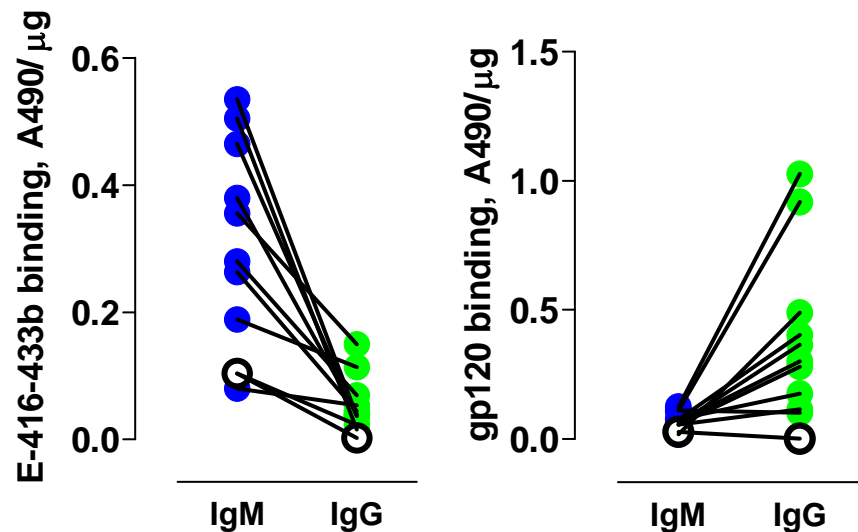
# The vulnerable but challenging CD4BS<sup>core</sup> - superantigenicity



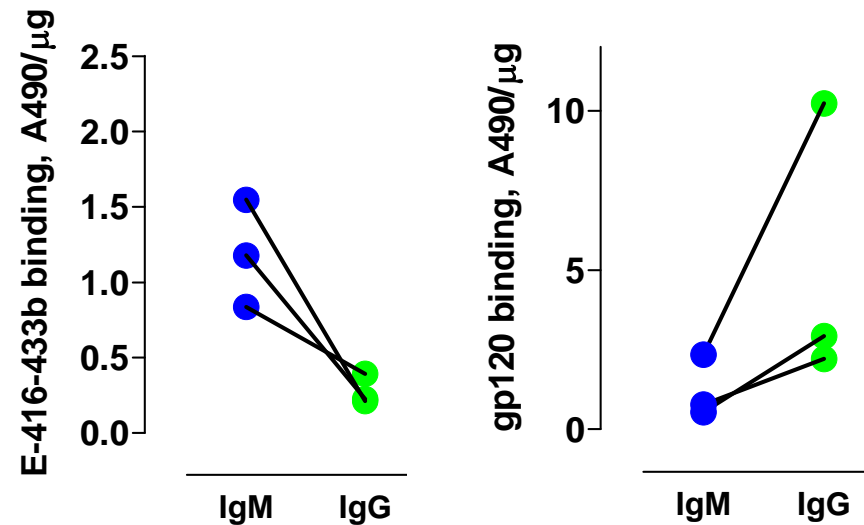
*Left*, Modest preimmune human Ab recognition of CD4BS<sup>core</sup> (neutralization+catalysis); Mouse/rabbit preimmune Abs recognize gp120 similarly; Others reported CD4BS<sup>core</sup> SAg character from binding studies

*Bottom*, Deficient IgM→IgG class switching [Abstract MOPE0045]

## HIV+ Subjects



## gp120 immunization



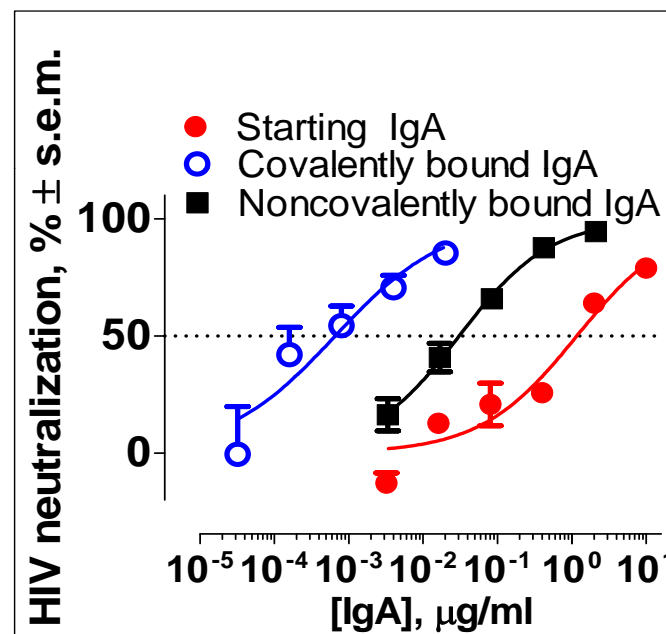
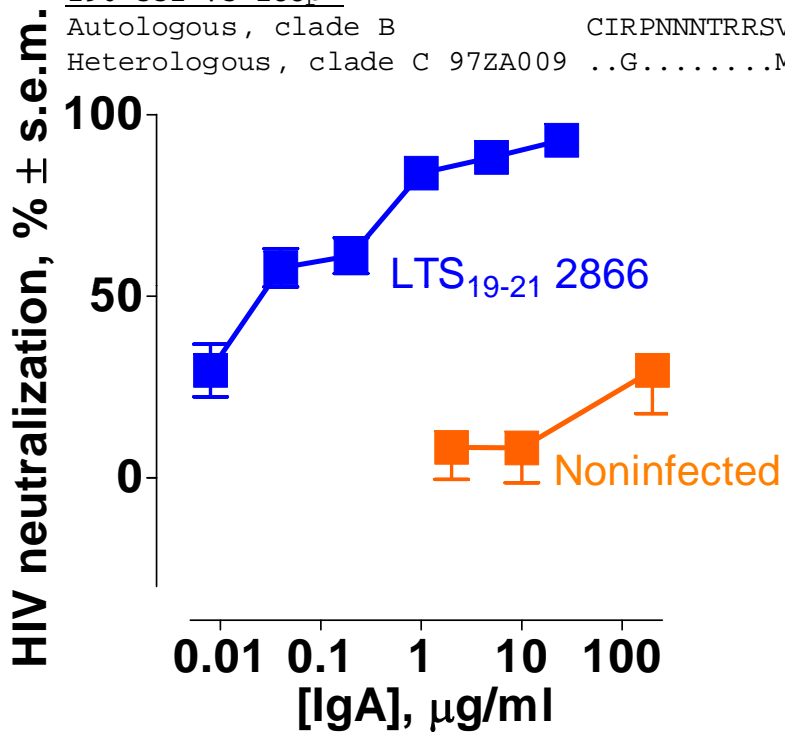
# CD4BS<sup>core</sup> vulnerability in long-term survivors

416-433 epitope:

Autologous, clade B .....  
 Heterologous, clade C 97ZA009 .....R.

296-331 V3 loop:

Autologous, clade B CIRPNNNTRRSVHIGPGSAFYTTGEIIGNIRQAHC  
 Heterologous, clade C 97ZA009 ..G.....MR....QV..A.NG.....



Left, Total IgA

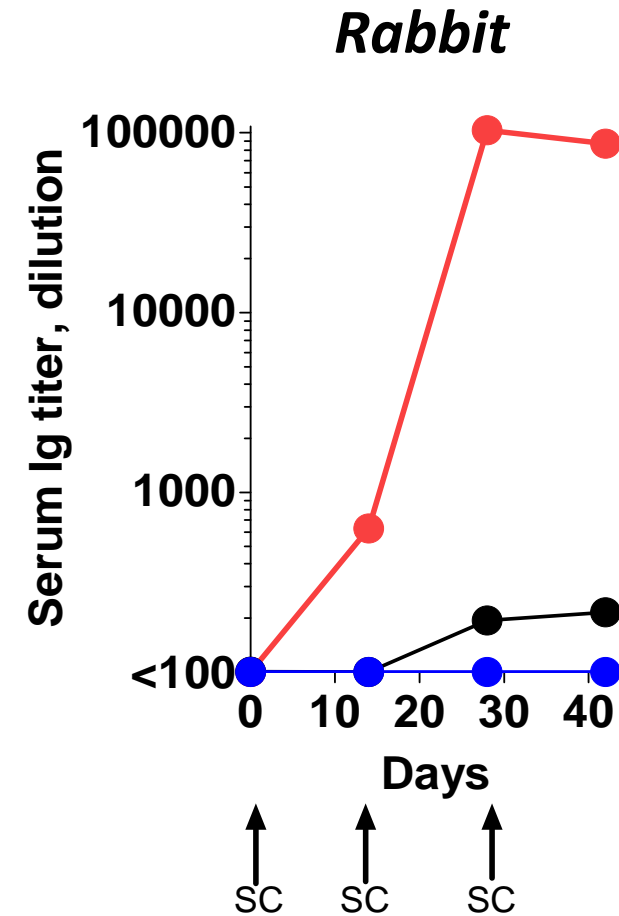
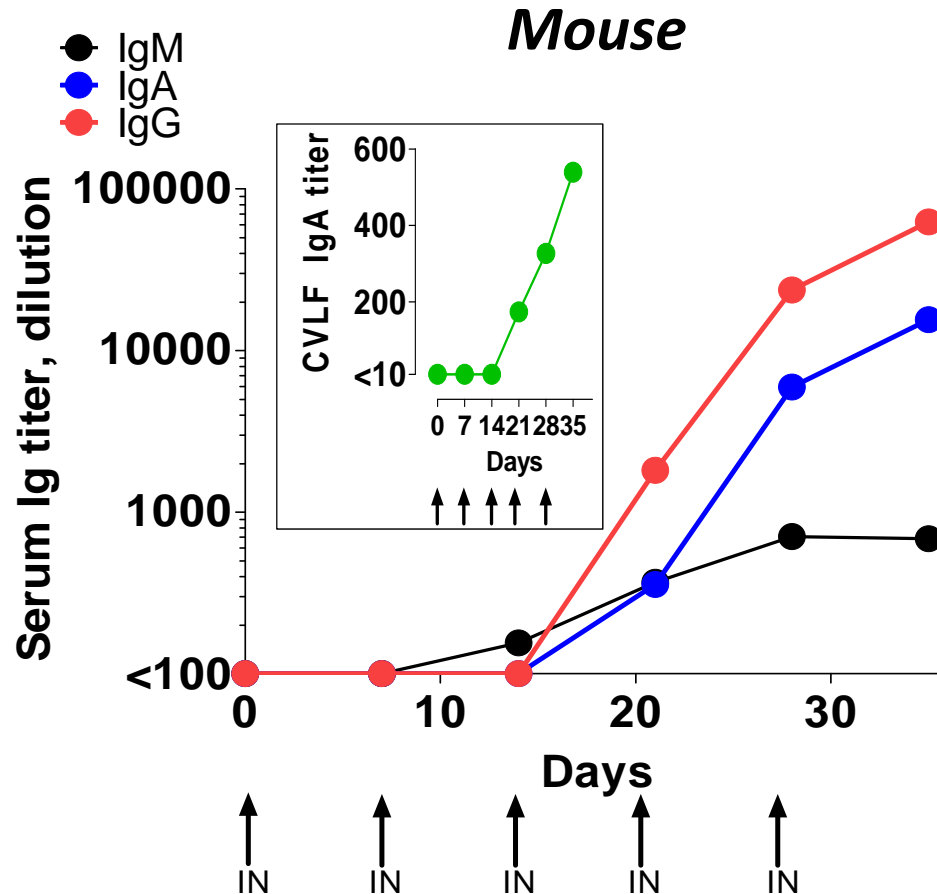
Right, CD4BS<sup>core</sup>-specific IgA

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# Polyclonal Abs raised by E-416-433 covalent immunization

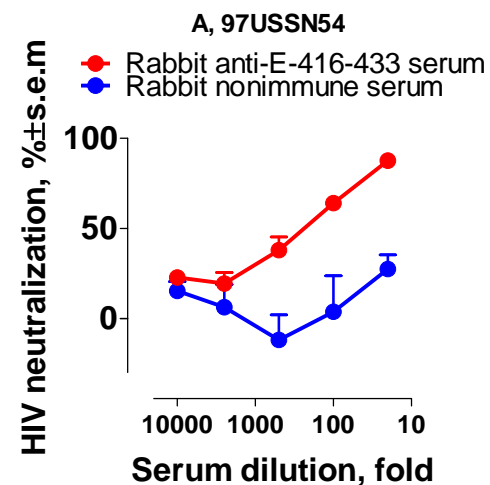
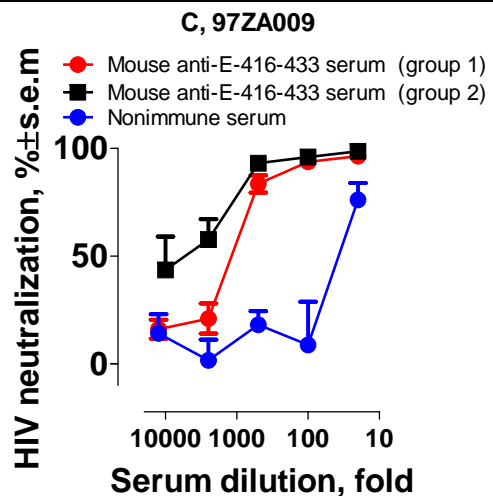
Covalent immunization of mice with full-length E-gp120 induced broadly neutralizing anti-CD4BS<sup>core</sup> IgG MAbs (Nishiyama JBC 2009) and rectified the problem of deficient IgG class switching (poster at this meeting).

To focus the Ab response at the CD4BS<sup>core</sup>, E-416-433 was employed as immunogen. Class-switched Ab responses were evident.

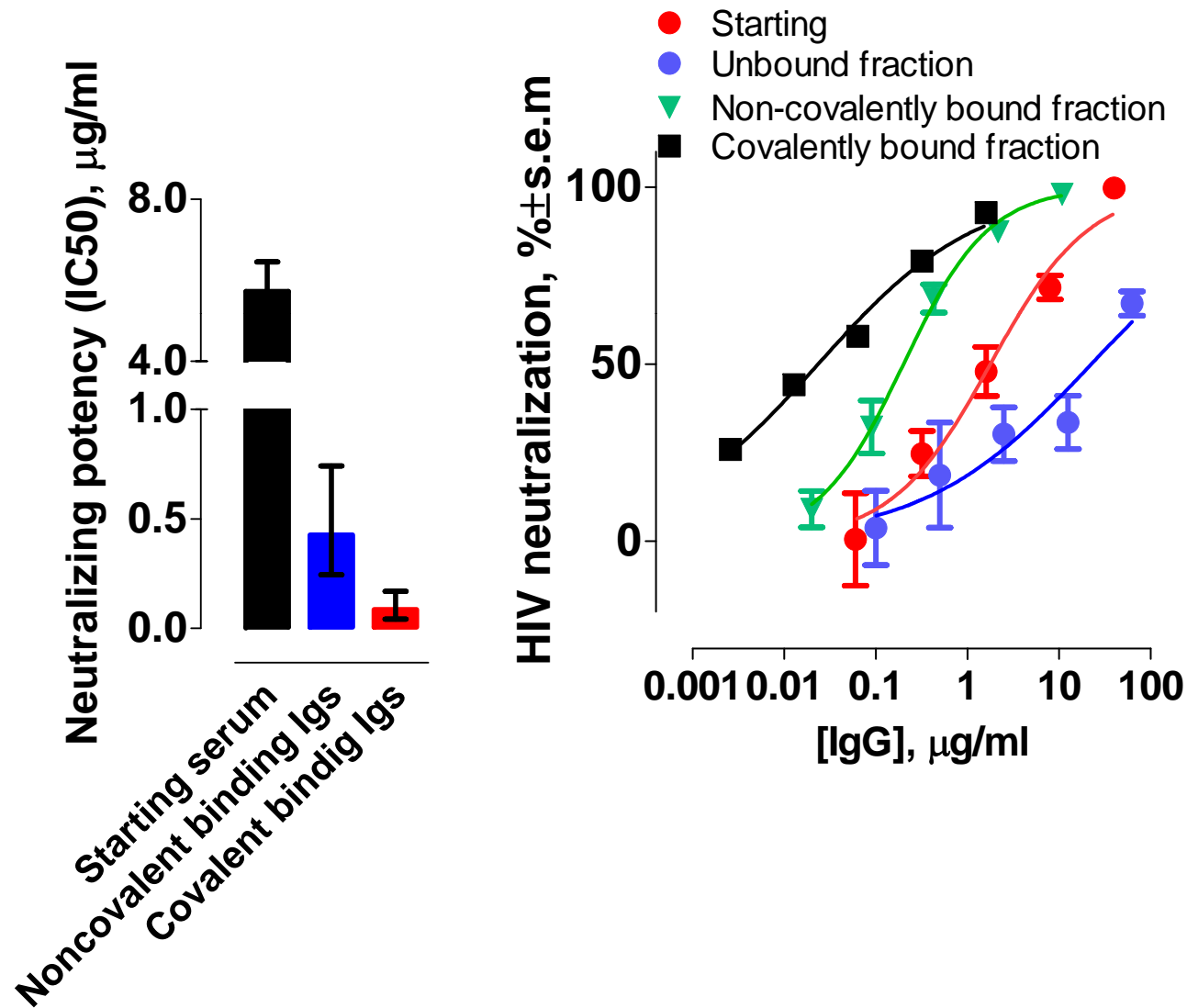


# Mouse and rabbit neutralizing polyclonal Abs raised by E-416-433 covalent immunization

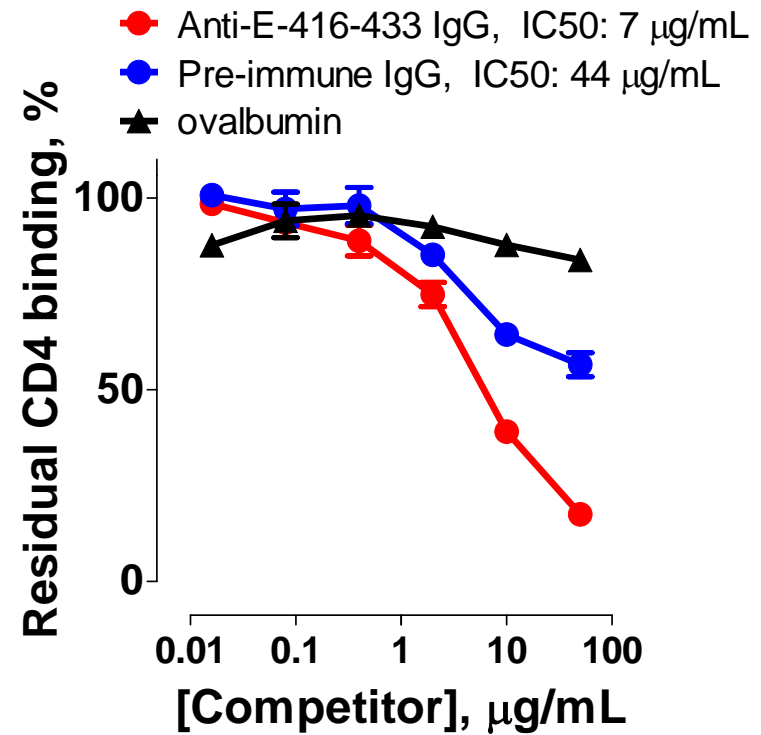
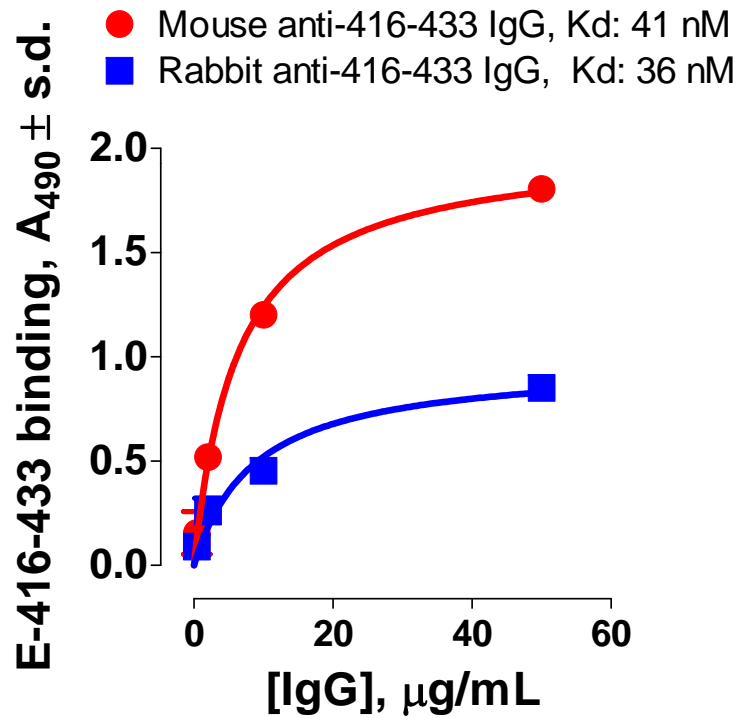
Subtype	Coreceptor	Strain	Titer		416-433 epitope sequence	V domain divergence, %				
			Rabbit	Mouse		V1	V2	V3	V4	V5
A	R5	97USSN54	296	NA	NA	NA	NA	NA	NA	NA
B	R5	92BR021	125	170	LPXRIKXIIIXMWQEVGKA	78	47	26	50	42
	R5	SHIV <sub>SF162P3</sub>	NA	101	LP <del>C</del> RIKQIINR <del>W</del> QEVGKA	79	47	21	50	62
	X4	92HT599	50	109	LP <del>C</del> RIKQIIV <del>N</del> MWQ <del>R</del> VGKA	91	53	47	69	50
C	R5	97ZA009	1714	1238	LP <del>C</del> RIKQIINMWQEVGRA					
	R5	98TZ017	61	NA	L <del>Q</del> CR <del>I</del> KQIINMWQEVG <del>R</del> A	81	39	21	62	62
	R5	98TZ013	NA	351	NA	62	40	18	NA	NA
D	R5	94UG114	160	61	L <del>Q</del> CR <del>I</del> KQIINMWQEVGKA	80	56	56	72	54
	X4	92UG001	50	NA	I <del>P</del> CR <del>I</del> KQIINMWQ <del>G</del> VGKA	90	44	36	63	31



# HIV neutralization by the induced Abs is due to E-416-433 recognition

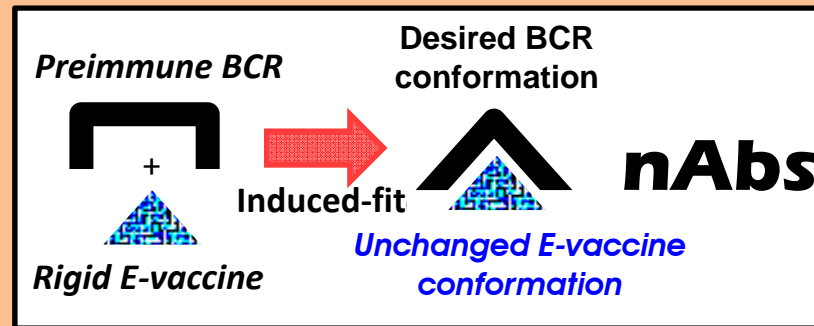


# CD4BS<sup>core</sup>-specificity of E-416-433 induced polyclonal IgG

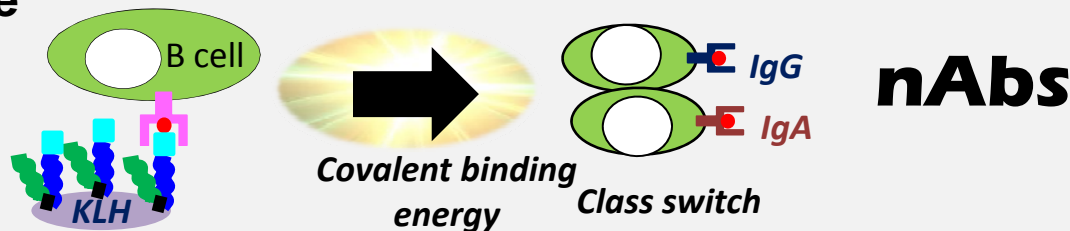


# Molecular determinants of vaccine efficacy

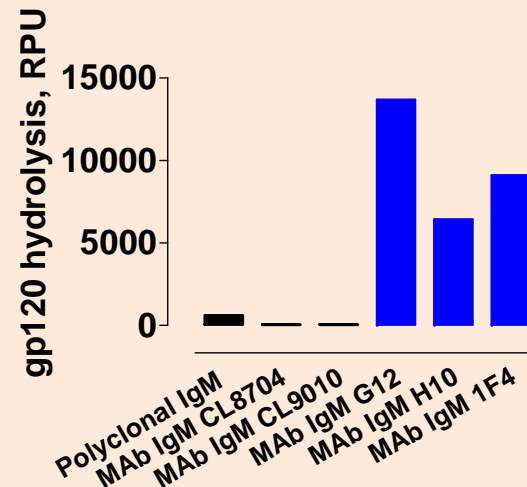
- Rigid, correct conformation of E-vaccine needed for adaptive neutralizing Ab synthesis



- Highly energetic B cell stimulation to bypass physiologically forbidden adaptive B cell response



- Bonus: An E-vaccine induced Ab subset catalyzes gp120 degradation rapidly, increasing vaccine efficacy



# Conclusions

- E-416-433 a conformationally correct CD4BS mimetic that binds CD4 autonomously.
- Unlike traditional immunogens, E-416-433 stimulates B cells covalently, bypassing the problem of B cell downregulation due to its superantigenicity, and inducing IgM → IgG/IgA class-switching required for effective protection.
- E-416-433 induced polyclonal neutralizing Abs in two animal species with the ability to neutralize genetically divergent strains.
- E-416-433 is the **first prototype vaccine** holding out the hope that a broadly neutralizing polyclonal Ab response to a conserved epitope can be induced for **globally effective HIV vaccination**.

Future direction: Improve neutralizing titers by optimizing the vaccination protocol (route, adjuvant, carrier protein).

# Acknowledgments

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