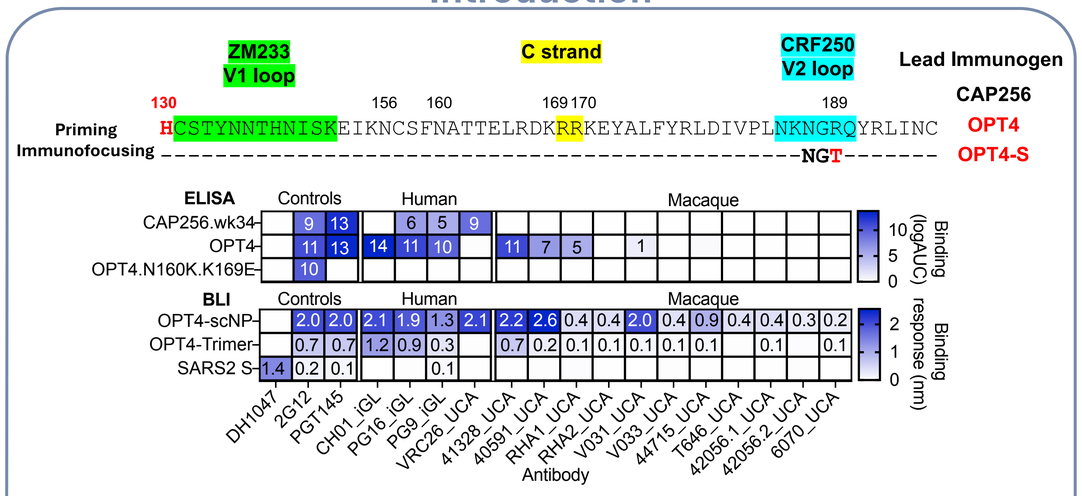
Enhanced B cell priming leads to accelerated and consistent V2 apex bNAb induction in SHIV-infected macaques: A molecular blueprint for immunogen design.

Lorie Marchitto[1*], Kshitij Wagh[2*], Ryan S. Roark[3*], Severin Coleon[2*], Hui Li[1], Ashwin N. Skelly[1], Michael P. Hogarty[1], Rumi Habib[1], Wenge Ding[1], Younghoon Park[1], Andrew J. Connell[1], Frederic Bibollet-Ruche[1], Samantha J. Plante[1], Macy J. Akeley[1], Jinery Lora[1] John W. Carey[1], Khaled Amereh[1], Xuduo Li[1], Raiees Andrabi[1], Edward F. Kreider[1], Drew Weissman[1], Robert J. Edwards[2], Lawrence Shapiro[3], Peter D. Kwong[3], Bette T. Korber[4], Barton F. Haynes[2], Kevin O. Saunders[2,10], Beatrice H. Hahn[1], George M. Shaw[1]

[1] Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA. [2] Duke University School of Medicine, Durham, NC, USA. [3] Aaron Diamond AIDS Research Center, Department of Biochemistry and Molecular Biophysics, Columbia University Vagelos College of Physicians and Surgeons, New York, NY, USA. [4] Sante Fe Institute, Los Alamos, NM, USA. *Authors contributed equally

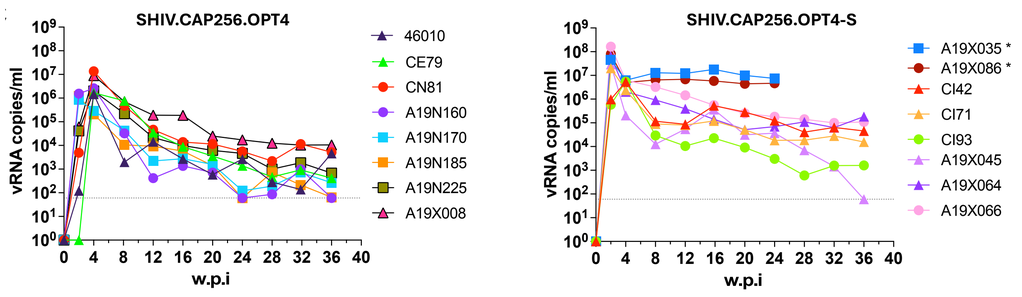
Introduction



We designed a novel germline-targeting Env, OPT4 and a minimal derivative, OPT4-S based on neutralization signature analysis.

- Testing designs in different Env backgrounds
- Design is limited to apex modifications
- Removal of the glycan at N130, introduction of natural loops, and testing of different C strand motifs

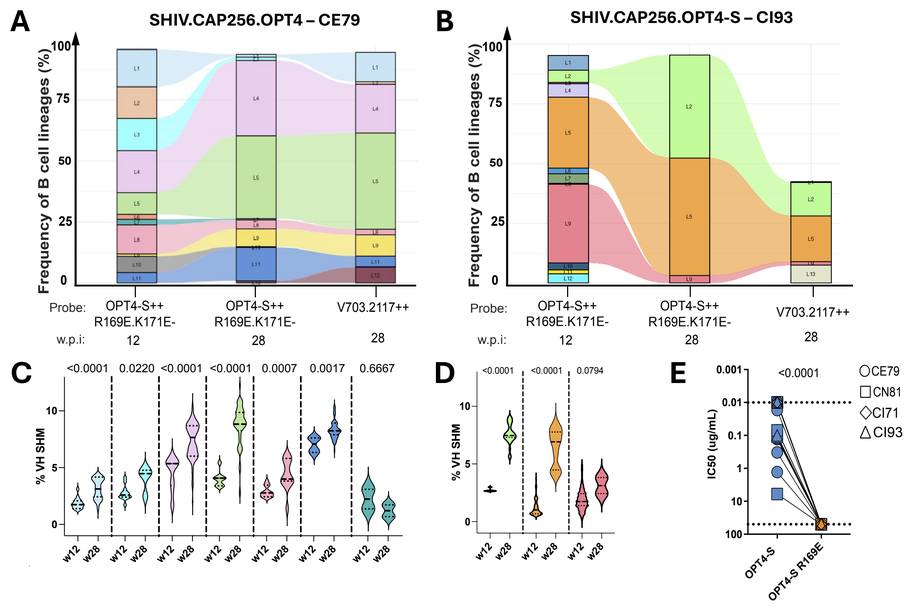
- **Selection based on V2 apex bNAb precursor binding; OPT4 bound multiple V2 apex UCAs or iGLs.**



We generated SHIV.CAP256.OPT4 and SHIV.CAP256.OPT4-S. Both SHIVs are replication competent and follow typical viral kinetic.

Two animals were rapid progressor and excluded from further analysis, leaving **14 evaluable animals**.
SHIV.CAP256.OPT4 n=8
SHIV.CAP256.OPT4-S n=6

Results



		SHIV.CAP256.OPT4			SHIV.CAP256.OPT4-S	
	mAb	CN81 2029	CE79 1571	CE79 1953	CI93 1043	CI93 1365
	MLV	>50	>50	>50	>50	>50
Autologous viruses	CAP256SU	0.03	>50	4	0.8	11
	CAP256.OPT4	<0.01	<0.01	<0.01	<0.01	<0.01
	CAP256.OPT4-S	<0.01	<0.01	<0.01	<0.01	<0.01
	CAP256.OPT4-S R169E	>50	>50	>50	>50	>50
	CAP256.OPT4-S N130	<0.01	0.02	<0.01	<0.01	<0.01
	CAP256.OPT4-S N130 R169E	>50	>50	>50	>50	>50
Viruses lacking N130	ZM233	0.3	>50	>50	23	12
	Q23	0.1	1	>50	<0.01	<0.01
	CAM13RRK	0.01	3	>50	0.024	<0.01
	C1080	0.1	>50	>50	>50	3
	CM244	10	0.03	24	13	0.2
	CE1176	<0.01	>50	0.2	25	>50
	CH119	>50	>50	>50	40	>50
	25710	>50	0.3	>50	>50	>50
	BJOX2000	>50	11	>50	23	0.3
	WITO	>50	>50	>50	>50	>50
	246F3	>50	2	>50	>50	0.1
	CE0217	10	>50	>50	>50	>50
	X2278	>50	>50	>50	>50	>50
	BG505	>50	14	>50	>50	>50
Viruses containing N130	TRO11	>50	>50	>50	>50	>50
	CH505	>50	>50	>50	6	0.3
	BF1266	0.2	>50	>50	>50	>50
	V703.2117	0.04	0.3	>50	<0.01	<0.01
	V703.2018	1	>50	>50	>50	>50
	V703.0629	>50	>50	>50	>50	>50
	V703.2149	0.04	>50	3	0.05	2
Breadth (%)	Heterologous viruses	52	38	14	48	48
	Viruses lacking N130	50	50	14	50	50
	Viruses containing N130	57	14	14	43	43

Fig. 3 – V2 apex bNAb lineages co-evolve and exhibit variable breadth and potency. A, B, Alluvial plots showing the longitudinal frequency of expanded B cell lineages with V2 apex bNAb-like immunogenetic characteristics sorted from peripheral blood 12 and 28 weeks post-infection. **C, D,** Percent of VH somatic hypermutation (SHM) in clonally related IgG+ B cells at 12 and 28 weeks post-infection in SHIV.CAP256.OPT4–infected animal CE79 (**C**) and SHIV.CAP256.OPT4-S–infected animal CI93 (**D**). Statistical significance was determined using Mann-Whitney tests. **E,** Neutralization potency (IC_{50} in μg/mL) of monoclonal antibodies isolated at 28 weeks post-infection against CAP256.OPT4-S and its V2 apex mutant R169E. Dashed horizontal lines represent the limit of detection. Statistical significance was determined using Wilcoxon signed-rank test. **F,** Neutralization potency (IC_{50} μg/mL) of representative bNAb lineage members isolated from three animals against the autologous V2 mapping panel and 21 heterologous viruses.

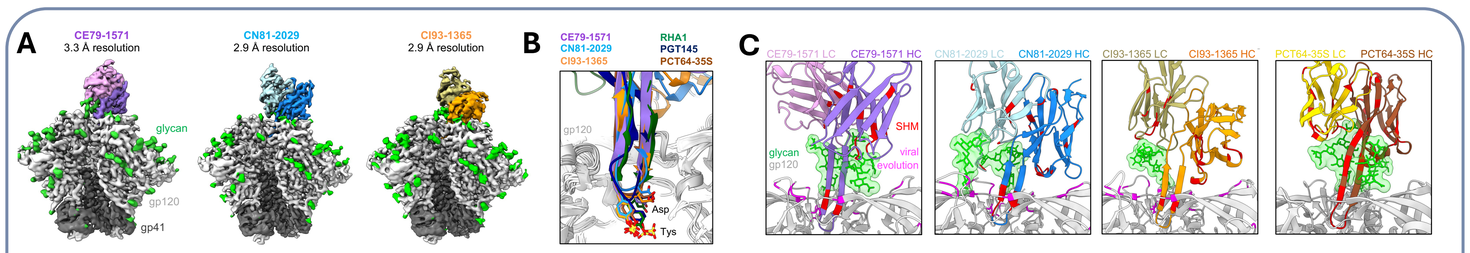


Fig. 4 – Cryo-EM structures of induced V2 apex bNAbs reveal needle-like recognition and critical SHM. **A**, Cryo-EM 3D reconstruction densities for identified bNAbs Fabs in complex with Q23-SCT27 trimer. The overall resolution is listed for each complex. **B**, Superimposed HCDR3 interface side view of the complex structures of identified bNAbs determined here aligned to envelope complexes with human Fabs PCT64-35S, PGT145 and rhesus Fab RHA1.V2.01(PDB-6XRT). Alignments were made with gp120 from each complex. Sulfated tyrosine residues (Tys) and adjacent Aspartate residues (Asp) are shown to highlight their conservation and positioning within the trimer. **C**, Expanded interface view of structures to highlight Fab binding position and HCDR3 insertion at the trimer apex. Apical envelope glycans bound by each respective Fab are shown in stick representation with transparent surfaces. Fab residues that underwent somatic hypermutation (SHM) from germline immunoglobulin sequences are colored in bright red. Envelope V1V2 residue positions that underwent complete mutational replacement in the viral quasispecies of each respective rhesus macaque subject by 28 weeks post-infection are colored in magenta. Glycan N160 is colored green.

RM ID	SHIV.CAP256.OPT4 CN81	A19N225	A19X008	A19N185	CE79	A19N170	A19N160	46010	SHIV.CAP256.OPT4-S A19X045	A19X066	CI93	A19X064	CI71	CI42
WPI	36	36	36	32	36	36	36	32	36	36	36	36	36	32
MLV	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
CAP256SU	1069	34	31	78	37	33	<20	<20	1765	333	117	234	<20	41
OPT4	647249	324359	373692	367918	85251	244499	359971	54230	231321	362713	379651	341297	125455	891266
OPT4-S	100614	36010	175500	278940	26357	26795	72886	255	24808	146649	66357	53163	26702	252461
OPT4-S R169E	8511	21017	83126	1044	2411	888	207	<20	19168	27307	2543	3540	2382	2613
OPT4-S N130	37147	10896	30276	36271	6527	3239	11191	<20	10812	54825	15598	10877	2146	27450
OPT4-S N130 R169E	11346	1095	9497	3956	2921	840	1077	21	5420	15540	2849	3580	826	5176
ZM233	1786	159	64	29	<20	21	<20	<20	121	98	113	68	24	34
Q23	485	4610	5705	19857	126	77	5238	<20	443	4792	5513	991	<20	148
CAM13RRK	221	3123	37	605	37	26	175	<20	121	221	3300	40	32	82
C1080	128	52	102	119	<20	<20	<20	<20	432	130	20	1839	<20	62
CM244	71	1329	42	45	23	158	<20	<20	2385	237	401	1179	26	38
CE1176	362	<20	34	<20	<20	<20	<20	<20	1031	59	89	<20	161	<20
CH119	20	<20	26	<20	<20	<20	<20	<20	27	152	38	<20	<20	<20
25710	30	104	<20	<20	<20	<20	<20	<20	212	26	<20	<20	<20	<20
BJOX002000	112	<20	<20	<20	<20	<20	<20	<20	47	302	79	21	<20	<20
WITO	25	61	43	<20	<20	<20	<20	<20	20	338	<20	<20	<20	<20
246F3	<20	56	<20	<20	<20	<20	<20	<20	71	217	<20	<20	<20	<20
CE0217	45	22	<20	<20	<20	<20	<20	<20	46	<20	34	<20	<20	<20
X2278	25	<20	33	<20	<20	<20	<20	<20	<20	32	<20	<20	<20	<20
BG505	33	40	<20	<20	<20	<20	<20	<20	53	37	<20	42	<20	<20
TRO11	46	<20	<20	<20	<20	<20	<20	<20	39	236	<20	<20	<20	<20
CH505	72	486	65	42	35	<20	<20	<20	238	600	541	56	67	<20
BF1266	136	<20	<20	<20	<20	<20	<20	<20	144	<20	<20	<20	<20	<20
V703.2117	651	308	136	7241	37	<20	2085	<20	83	3361	806	54	<20	215
V703.2018	55	24	<20	<20	<20	<20	<20	<20	77	237	<20	<20	<20	<20
V703.0629	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
V703.2149	165	57	120	26	<20	<20	<20	<20	313	269	75	22	35	<20
Breadth (%) Heterologous viruses	90	67	57	38	24	19	14	0	90	86	57	48	29	29
Viruses lacking N130	93	71	64	36	21	29	14	0	93	93	64	50	29	36
Viruses containing N130	86	57	43	43	29	0	14	0	86	71	43	43	29	14

ID_{50} <20 | ID_{50} [20 – 99] | ID_{50} [100 – 999] | ID_{50} [1,000 – 9,999] | ID_{50} [10,000 – 99,999] | ID_{50} [100,000 – 900,000]

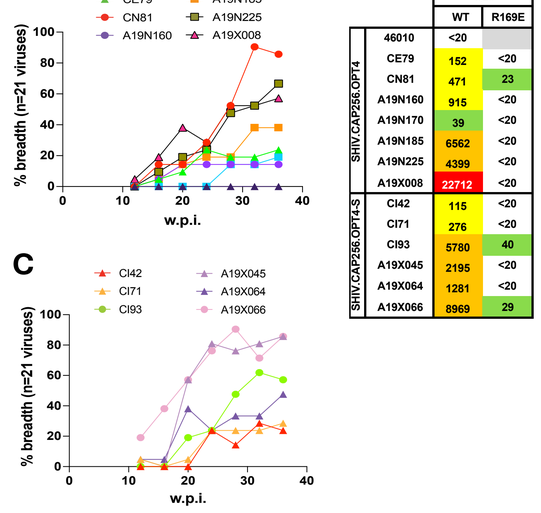


Fig. 1 – 93% of RMs developed C-strand directed heterologous neutralization breadth. A, Plasma neutralization of the indicated viruses at the timepoint of greatest breadth within 36 weeks of infection is shown for each animal, expressed as reciprocal ID_{50} titers. **B, C,** Longitudinal heterologous plasma neutralization breadth on a 21-virus panel in animals infected with SHIV.CAP256.OPT4 (**B**) or SHIV.CAP256.OPT4-S (**C**). **D,** Heterologous plasma neutralization activity at 28 weeks post-infection against Env Q23 and its V2 apex mutant (Q23.R169E) expressed as reciprocal ID_{50} titer. w.p.i., weeks post-infection.

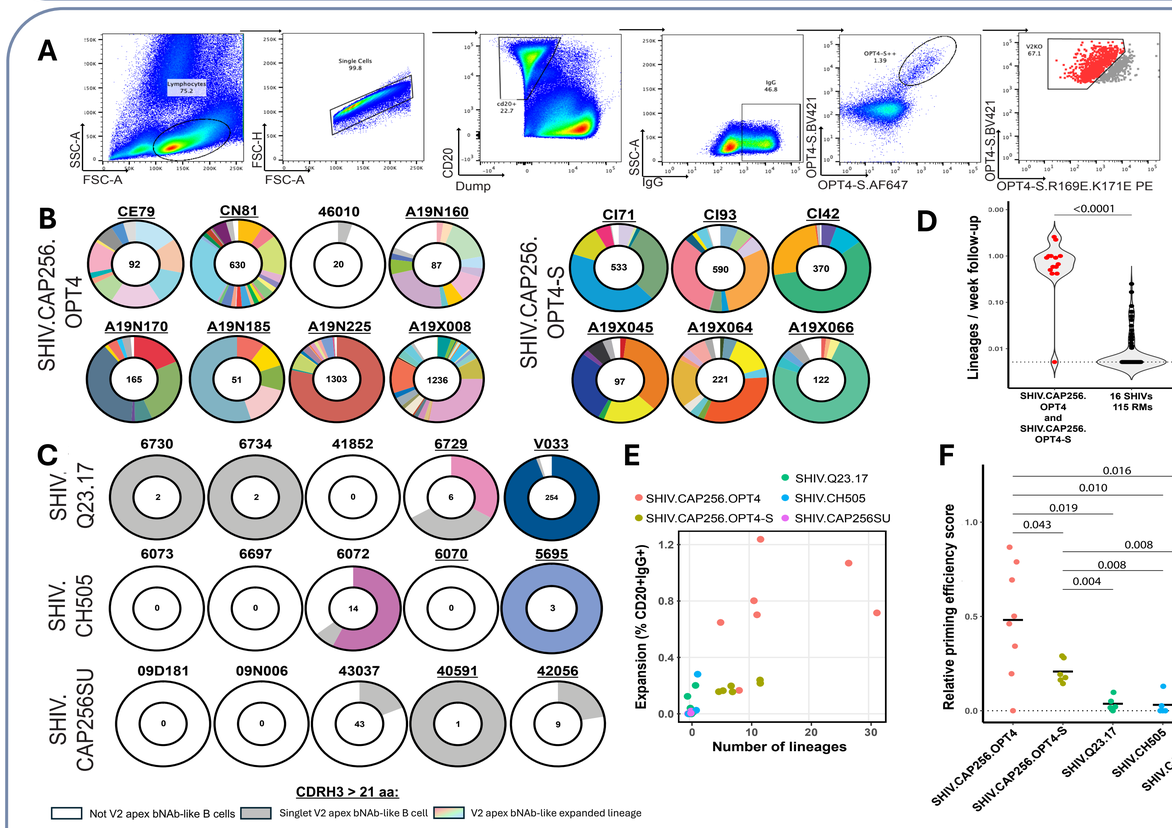


Fig. 5 – Env-bNAb coevolution in SHIV.CAP256.OPT4 and OPT4-S infected macaques. A, Longitudinal logo plots spanning V1 (130-132) and V2 sites (154-184) showing mutations away from the OPT4-S sequence. "O" represents an Asn in a N-linked glycosylation sequon. The height of each letter corresponds to the frequency of that amino acid in the longitudinal sample. Chemistry based color scheme is used: blue indicates positively charged basic amino acids, red indicates negatively charged acidic amino acids, black indicates hydrophobic amino acids, green indicates non-polar amino acids and purple indicates neutral amino acids. **B,** Median hypervariable V1 (blue) and V2 (cyan) length, glycan content and net charge. Black bars span inter-quartile range. Right panel - serum breadth.

Fig. 2 – SHIV.CAP256.OPT4/S increase priming of V2 apex bNAb-like B cells by 40-370-fold compared to wildtype Envs. A, Representative gating strategy used to isolate IgG+ epitope-specific V2 apex B cells from peripheral blood 12 weeks post-infection; red dots represent sorted cells. **B, C,** Pie charts show the clonal diversity and expansion of peripheral IgG+ B cells isolated 12 weeks post-infection. Animals that developed V2 apex bNAbs based on plasma neutralization at any given time after SHIV infection are underlined. **D,** Number of primed B cell lineages with immunogenetic features of V2 apex bNAbs per week of follow-up. **E,** Scatter plot comparing the priming of V2 apex-targeted B cell lineages in macaques infected by different SHIVs. Each dot represents a macaque, colored by SHIV strain. **F,** Relative priming efficiency of V2 apex B cells 12 weeks post-infection in animals infected with SHIV.CAP256.OPT4 or SHIV.CAP256-OPT4-S compared with animals infected with SHIVs described to have a propensity to induce V2 apex bNAbs.

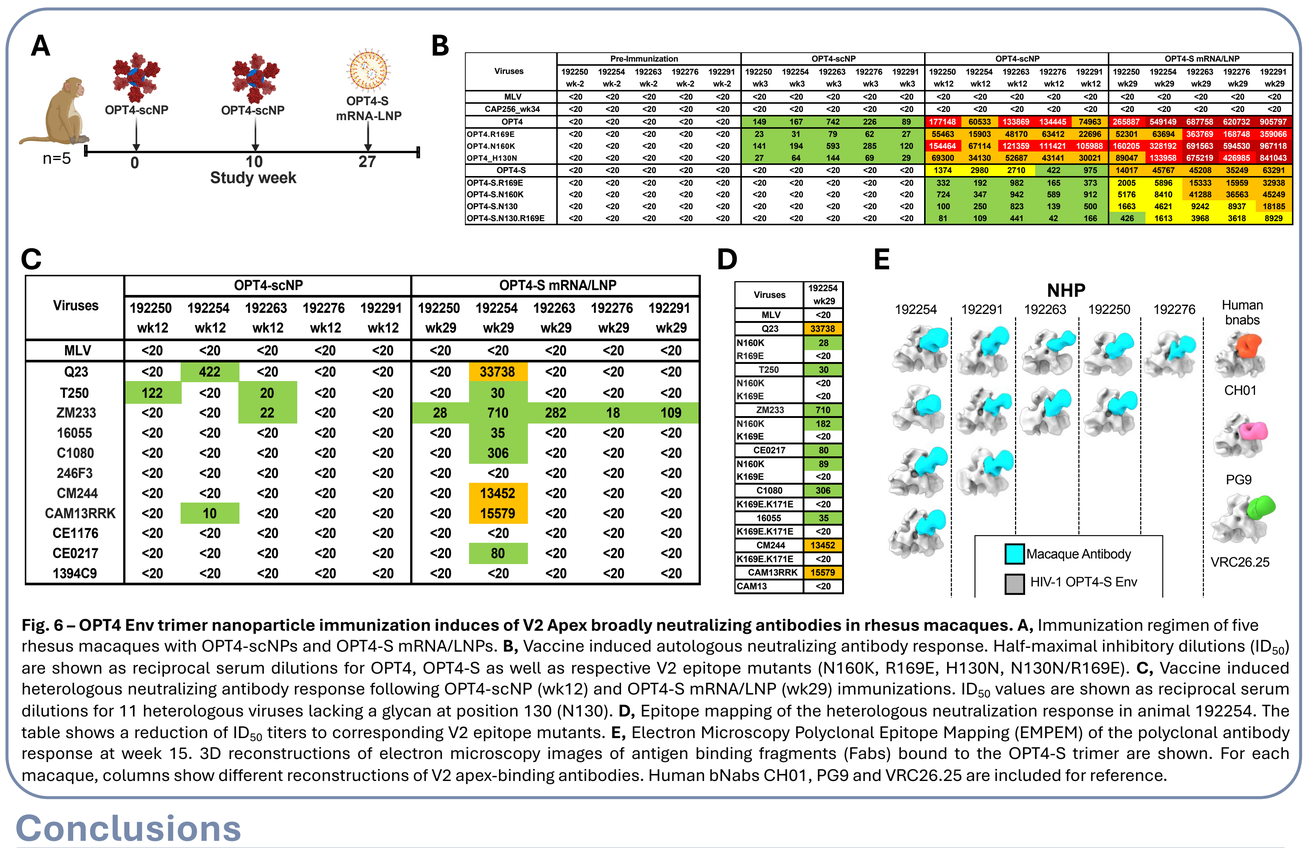


Fig. 6 – OPT4 Env trimer nanoparticle immunization induces of V2 Apex broadly neutralizing antibodies in rhesus macaques. A, Immunization regimen of five rhesus macaques with OPT4-scNPs and OPT4-S mRNA/LNPs. **B,** Vaccine induced autologous neutralizing antibody response. Half-maximal inhibitory dilutions (ID_{50}) are shown as reciprocal serum dilutions for OPT4, OPT4-S as well as respective V2 epitope mutants (N160K, R169E, H130N, N130N/R169E). **C,** Vaccine induced heterologous neutralizing antibody response following OPT4-scNP (wk12) and OPT4-S mRNA/LNP (wk29) immunizations. ID_{50} values are shown as reciprocal serum dilutions for 11 heterologous viruses lacking a glycan at position 130 (N130). **D,** Epitope mapping of the heterologous neutralization response in animal 192254. The table shows a reduction of ID_{50} titers to corresponding V2 epitope mutants. **E,** Electron Microscopy Polyclonal Epitope Mapping (EMPEM) of the polyclonal antibody response at week 15. 3D reconstructions of electron microscopy images of antigen binding fragments (Fabs) bound to the OPT4-S trimer are shown. For each macaque, columns show different reconstructions of V2 apex-binding antibodies. Human bNAbs CH01, PG9 and VRC26.25 are included for reference.

Conclusions

- **93% of macaques infected with SHIV.CAP256.OPT4 or OPT4-S developed V2 apex bNAbs within 36 weeks of infection. bNAb priming occurred as early as 4 weeks post-infection and bNAbs became detectable in plasma as early as 12 weeks.**
- **SHIV.CAP256.OPT4 and OPT4-S primed V2 apex bNAb-like precursors 40 to 370-fold more efficiently than did SHIVs expressing wildtype HIV-1 Envs.**
- **Isolated bNAbs showed highly conserved needle-like trimer binding, similar to human bNAbs PCT64 and PGT145.**
- **Env-Ab coevolution revealed common V1/V2 substitutions and loop changes leading to breadth, thus informing the design of boost immunogens for future vaccine studies including human trials currently underway (HVTN322).**
- **Pilot study in 5 rhesus macaques show heterologous neutralization with titers as high as 1:34,000 and a GMT of 1:774.**

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CHAVD Duke Consortia for HIV/AIDS Vaccine Development

NIH National Institute of Allergy and Infectious Diseases (NIAID)

NIH National Institute of Allergy and Infectious Diseases

Division of AIDS (DAIDS)