HIV-1 Vaccine

Presented by Duo Li

4 February 2014

BACKGROUND

Overview: HIV-1 vaccine approaches

induce **antibodies**, especially broadly neutralizing

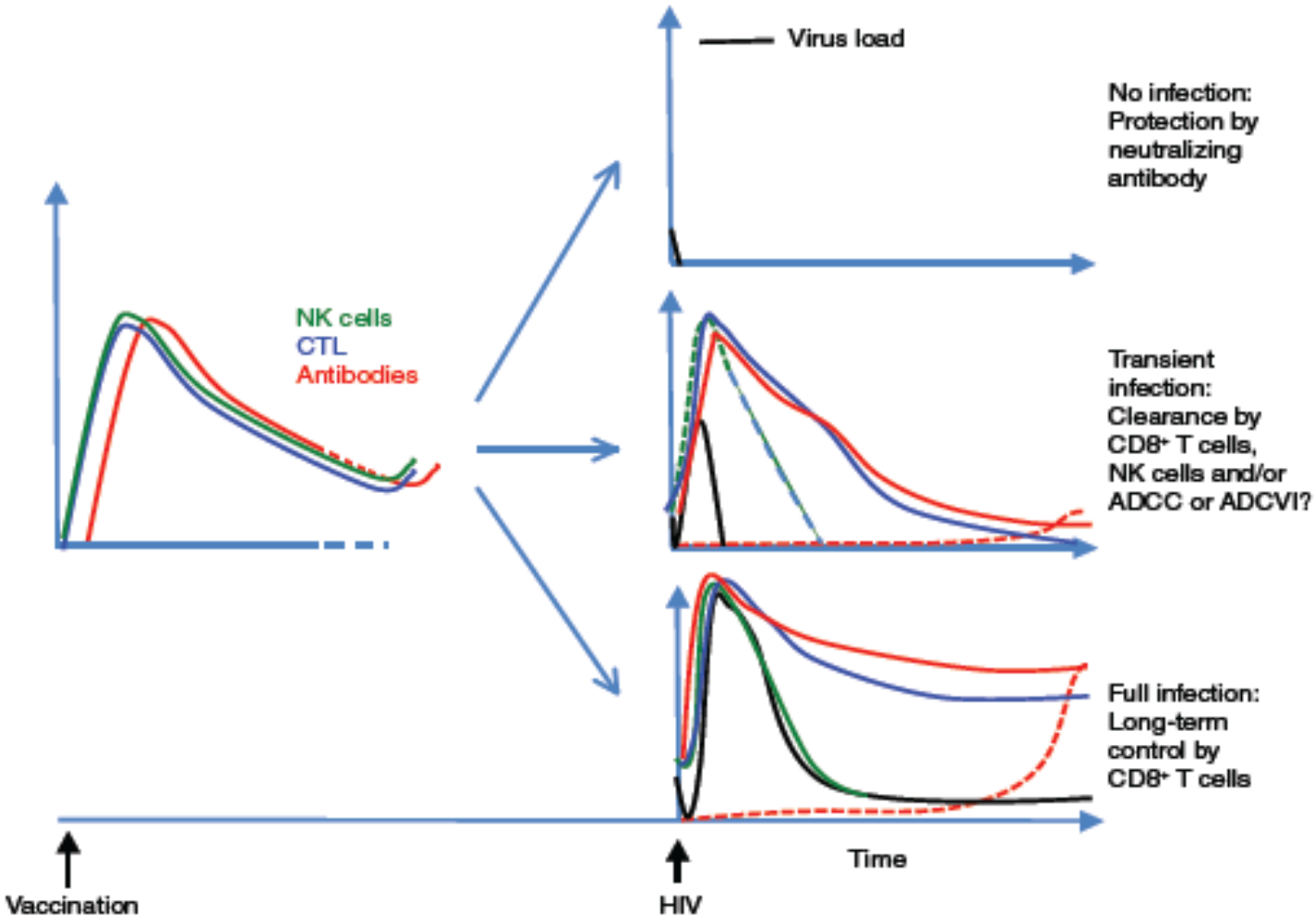
induce responsive CD4+ and/or CD8+ **T cells**

design immunogen containing epitopes recognized by T cells when presented by given HLA molecules

modulate natural killer cells, regulatory T cells, etc.

Reference: McMichael & Haynes, *Nat Immunol* ,(2012)

Three possible protective outcomes of an HIV-1 vaccine



Reference: McMichael & Haynes, *Nat Immunol* ,(2012)

A brief history of HIV-1 vaccines

- **1984**: prediction that vaccine against HIV available within 2 years [1]
- by **2008**: already >50 candidate vaccines reached clinical trials [2]
- few reached large-scale testing (phase III) [2,3]
  - AIDSVAX B/E
  - STEP (MRK rAd5)
  - RV144 (prime + boost):
- ongoing work to understand why RV144 showed efficacy and to design improved vaccines [3]

2012: "There is now considerable new optimism in the HIV-1 vaccine-development field because of the remarkable progress in research over the past 3 years." [3]

References:
[1] Editorial, *Lancet*, (2009); [2] McElrath *et al*, *Lancet* , (2008); [3] McMichael & Haynes, *Nat Immunol* ,(2012)

HIV vaccine approaches and efficacy trials

Table 1. HIV vaccine efficacy trials.

	Efficacy trial	Vaccine	Population	*N*	Efficacy	Other significant results	Immune response	Immune correlates of risk	
Protein subunit vaccines	VAX004	AIDSVAX B/B (rgp120 immunogens)	Primarily high-risk MSM	5403	None [Flynn *et al.* 2005]	N/A	Weak nAb response [Gilbert *et al.* 2010]	N/A	North America, Netherlands
	VAX003	AIDSVAX B/E (rgp120 immunogens)	Injection drug users	2546	None [Pitisuttithum *et al.* 2006]	N/A	Weak nAb response [Montefiori *et al.* 2012]	N/A	Thailand
Viral vector vaccines	Step	MRKAd5 HIV-1 (rAd5 vector expressing Gag, Pol, and Nef)	Primarily high-risk MSM	3000	None; trial halted after meeting prespecified futility boundaries [Buchbinder *et al.* 2008]	Significantly increased risk of HIV infection in men who were both Ad5-seropositive and uncircumcised, which waned with time since vaccination [Duerr *et al.* 2012]	CD8+ T-cell response detected in the majority of vaccinees, although weak and of narrow breadth [McElrath *et al.* 2008]	N/A	North and South America the Caribbean, Australia
	HVTN 503/ Phambili	Same as Step	Primarily heterosexuals	801	None; enrollment halted after lack of efficacy seen in Step [Gray *et al.* 2011b]	N/A	Similar to Step [Gray *et al.* 2011b]	N/A	
Heterologous prime-boost regimens	RV144	ALVAC (canarypox vector expressing Env, Gag, and Pol) prime followed by AIDSVAX B/E boost	Primarily low-risk heterosexuals	16,402	31.2% overall efficacy for prevention of HIV-1 infection in the modified intention-to-treat analysis [Rerks-Ngarm *et al.* 2009]; no subsequent effect on viremia or CD4 count in vaccinees who were infected [Rerks-Ngarm *et al.* 2012]	68% efficacy for low or medium risk participants, no efficacy in the high-risk group; efficacy was highest over the first 12 months and then fell rapidly [Robb *et al.* 2012]	Weak nAb response [Montefiori *et al.* 2012]. Moderate CD8+ and CD4+ T- cell response; the CD4+ T-cell response was directed against the V2 region of Env [de Souza *et al.* 2012]	Binding of IgG to the V1 and V2 regions of Env correlated with protection; protection was mitigated by the presence of plasma IgA directed against Env [Haynes *et al.* 2012a]	Thailand
DNA vaccines	HVTN 505	VRC-HIVDNA016-00-VP (DNA expressing Gag, Pol, Nef, and Env) prime followed by VRC-HIVADV014-00-VP (rAd5 expressing Gag, Pol, and Env) boost	High-risk MSM	2504	None; trial halted after meeting prespecified futility boundaries	Awaiting final trial results	Awaiting final trial results	N/A	United States

HIV, human immunodeficiency virus; HVTN, HIV Vaccine Trials Network; IgG, immunoglobulin G; MSM, men who have sex with men; nAb, neutralizing antibody; rgp, recombinant glycoprotein; rAd5, recombinant adenovirus serotype 5.

Reference: Cohen *et al.*, *Ther Adv Vaccines* (2013)

Current vaccine challenges & strategies

(both antibody- and T cell-based vaccines)

An effective vaccine needs to…

- closely match sequences of circulating HIV-1
- account for the variability of circulating HIV-1 strains
- avoid immune escape
- promote vaccine-induced responses over more dominant but less effective natural immune responses

References: McMichael & Haynes, *Nat Immunol* ,(2012)

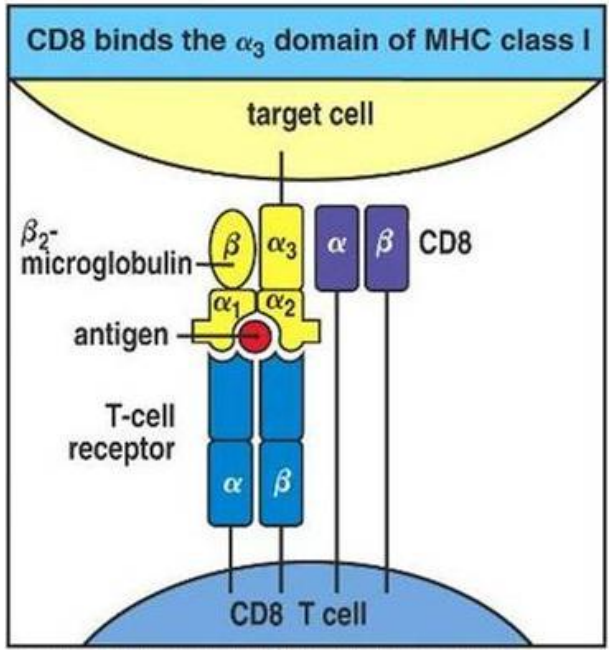
Strategies to deal with HIV-1 diversity

reviewed by Letourneau *et al.* (2007)

Immunogen design	Advantages/Support	Disadvantages
Single natural isolate	• simple • many reports of cross-clade reactive T cell responses	• unrealistically high peptide concentration •unlikely to provide sufficient protection
"Centralized" sequences	• evidence of immunogenicity and T cell response in small-scale animal & human trials	• "may be stretched too far for optimal coverage"
Cocktail of immunogens from multiple clades	• promising initial results with responses to all antigens in cocktail	• potential for immune interference
"Mosaic" immunogen designed computationally	• based on intact proteins → can be naturally processed and presented	• potential for immune interference • potential that responses to variable regions detract from responses to conserved regions

Immunodominant vs. subdominant

- **Immunodominant:** determinants are recognized by the **most abundant** cognate T cell populations.
- **Subdominant:** determinants are recognized by **less abundant** T cell populations.
- Dominant responses limit responses to subdominant antigens.



Vaccines targeting conserved regions

Advantages:

- highly conserved sub-protein regions are common to most HIV-1 strains
- immune escape mutations in conserved regions are costly

Challenges:

- immune responses to conserved regions tend to be subdominant
  - ➢ must compete with faster-changing dominant epitopes for both T-cell priming and target recognition

But: "it should be possible to re-direct CD8+ T-cell responses to focus on particular conserved epitopes"

References: McMichael & Haynes, *Nat Immunol* ,(2012); Letourneau *et al. , PLoS ONE* ,(2007); M. Altfeld & T. M. Allen, "Hitting HIV where it hurts: an alternative approach to HIV vaccine design", *Trends Immunol*,(2006).

Timeline of HIVconsv vaccine development

group of Tomas Hanke (Oxford)

Publication date			*References*
2007	Vaccine design, preliminary testing	* immunogen design * expression in human cells * immunogenicity in BALB/c mice * presence of responses in natural HIV-1 infection	Letourneau *et al. PLoS ONE* 2:e984 (2007)
2010-2012	Efficacy in animal models	* confirm immunogenicity in mice and macaques * optimize delivery regime * other vaccine types also highly effective!	Rosario *et al., Eur J Immunol* 40:1973-1984 (2010) Knudsen *et al., J Virol* 86:4082-4090 (2012) Rosario *et al., AIDS* 26:275-284 (2012)
2013	Safety in animal models	* intramuscular injection of all 3 vector-delivered vaccines well-tolerated in BALB/c mice	Ondondo *et al.*, *Vaccine*, in press (2013)
2013	First human trial (phase I clinical trial)	* test safety and efficacy in HIV-1/2- uninfected human volunteers	Borthwick *et al., Molecular Therapy*, accepted (2013)

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PLoS one

Design and Pre-Clinical Evaluation of a Universal HIV-1 Vaccine

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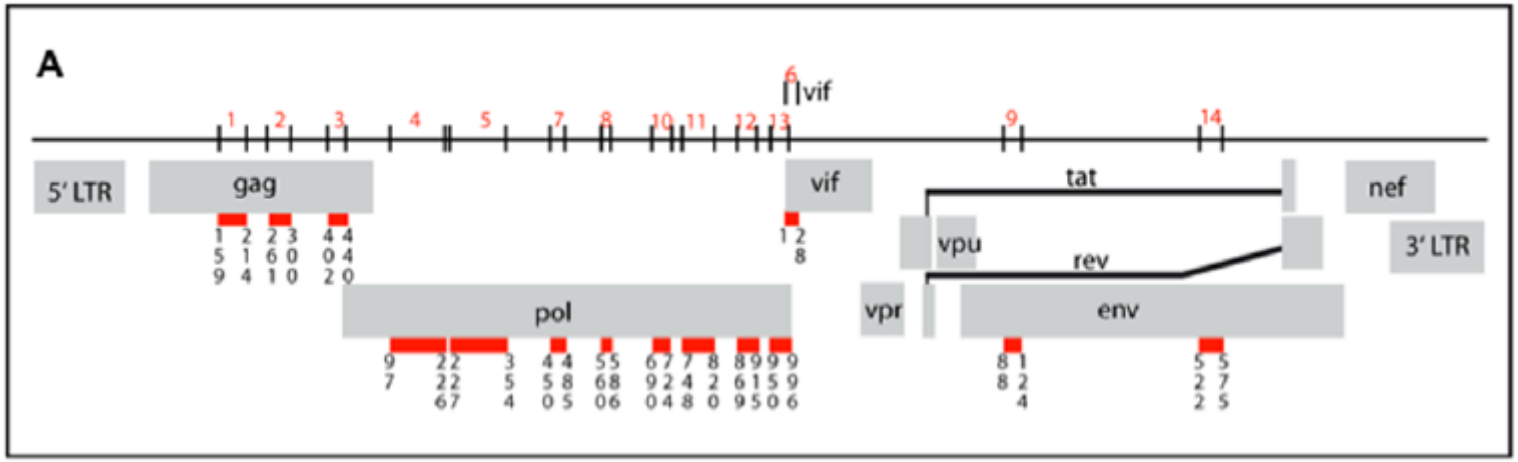
VACCINE DESIGN, PRELIMINARY TESTING

Main message:

- Vaccine tested: pTH. HIV consv, AdHu5.HIVconsv, MVA.HIVconsv
- Detect HIVconsv protein by immuno histochemical stainning
- Test immunogenicity in BALB/c mice, transgenic mice HLA-A2restricted, PBMC sample from HIV-1 infected patients

Design of the HIVconsv immunogen



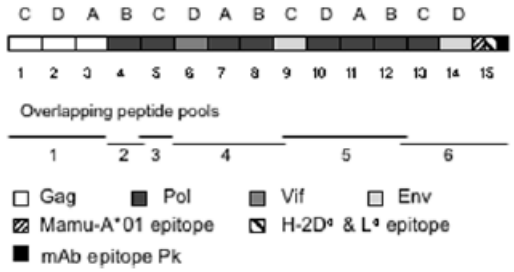
B

1 MEEKAFSPEVIPMFTALSEGATPQDLNTMLNTVGGHQAAMQMLKDTINE EAAEWDR
2 IYKRWIILGLNKIVRMYSPVSILDIRQGPKEPFRDYVDRF
3 ARNCRAPRKKGCWKCGKEGHQMKDCTERQANFLGKIWPS
4 RWKPKMIGGIGGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQI GCTLNFPISPIETVPVKLKPGMDGPKVKQWPLTEEKIKALVEICTEMEKEG KISKIGPENPYNTPVFAIKKKDSTKW
5 RKLVDFRELNKRTQDFWEVQLGIPHPAGLKKKKSVTVLDVGDAYFSVPL DEGFRKYTAFTIPSINNETPGIRYQYNVLPQGWKGSPAIFQSSMTKILEPF RAQNPEIVIYQYMDDLYVGSDLEIGQHR
6 MENRWQVMIVWQVDRMRIRTWKSLVKHH
7 LTEEAELELAENREILKDPVHGVYYDPSKDLIAEIQ
8 YWQATWIPEWEFVNTPPLVKLWYQLEK
9 NVTENFNMWKNDMVDQMHEDIISLWDQSLKPCVKLTP
10 WVPAHKGIGGNEQVDKLVSQGIRKVLFLDGIDKAQ
11 AKEIVASCDKCQLKGEAMHGQVDCSPGIWQLDCTHLEGKVILVAVHVAS GYIEAEVIPAETGQETAYFLLKLA
12 MNKELKKIIGQVRDQAEHLKTAVQMAVFIHNFKRKGGIGGYSAGERI
13 WKGPAKLLWKGEGAVVIQDNSDIKVVPRRKAKIIRDYGKQMAGADCV
14 FLGAAGSTMGAASMTLTVQARQLLSGIVQQQNNLLRAIEAQQHLLQLTV WGIKQ
15 ACTPYDINQMLRGPGRAFVTIPNPLLGLD

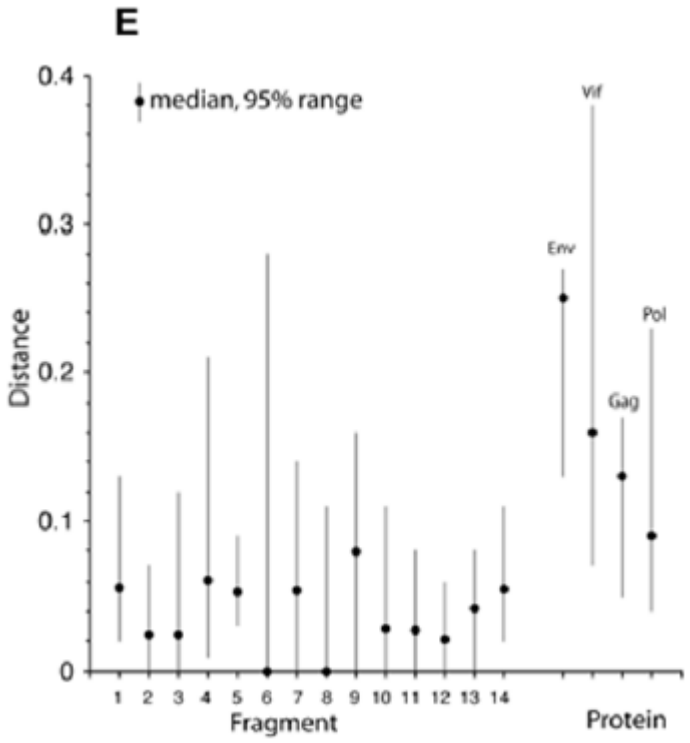
C

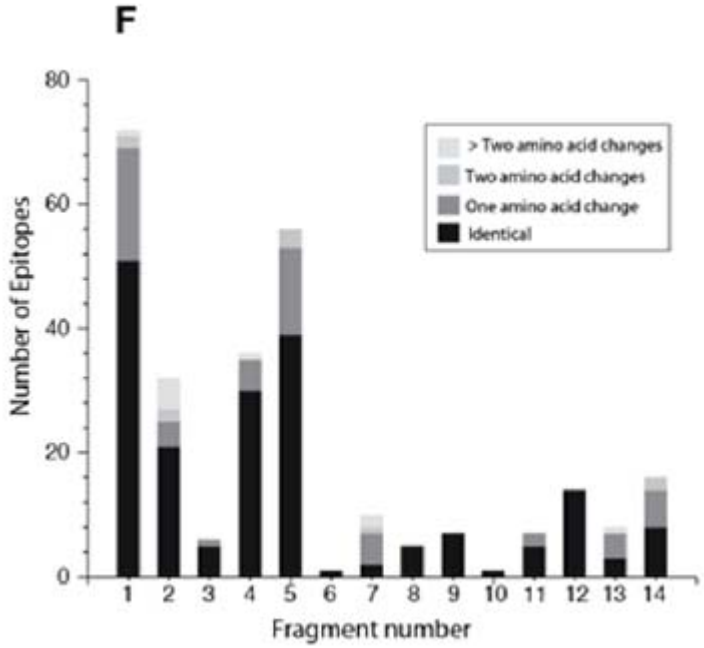
Fragm	Protein	Clade	Identical consensus	HIV_{CONSV} (aa)
1	Gag	C	A2, CRF08	1-56
2	Gag	D	M, A2, F, G, K, CRF01	57-96
3	Gag	A	M, C, G, CRF01	97-135
4	Pol	B		136-265
5	Pol	C		266-393
6	Vif	D	B	394-421
7	Pol	A		422-457
8	Pol	B	M, A, C, D, F, CRF01, CRF02, CRF08	458-484
9	Env	C		485-521
10	Pol	D		522-556
11	Pol	A		557-629
12	Pol	B	M, A2, C, D, F, G	630-676
13	Pol	C	CRF08	677-723
14	Env	D	B	724-777
15	Added macaque, mouse and mAb epitopes			778-806

D



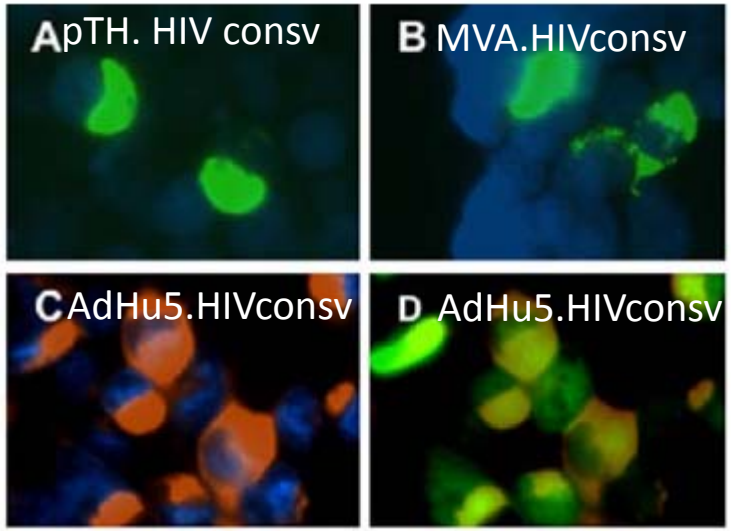
Design of the HIVconsv immunogen



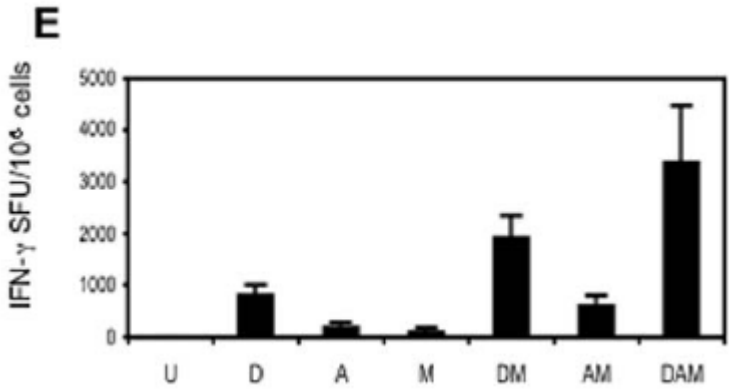


HXB2

Vaccine construction and basic immunogenicity



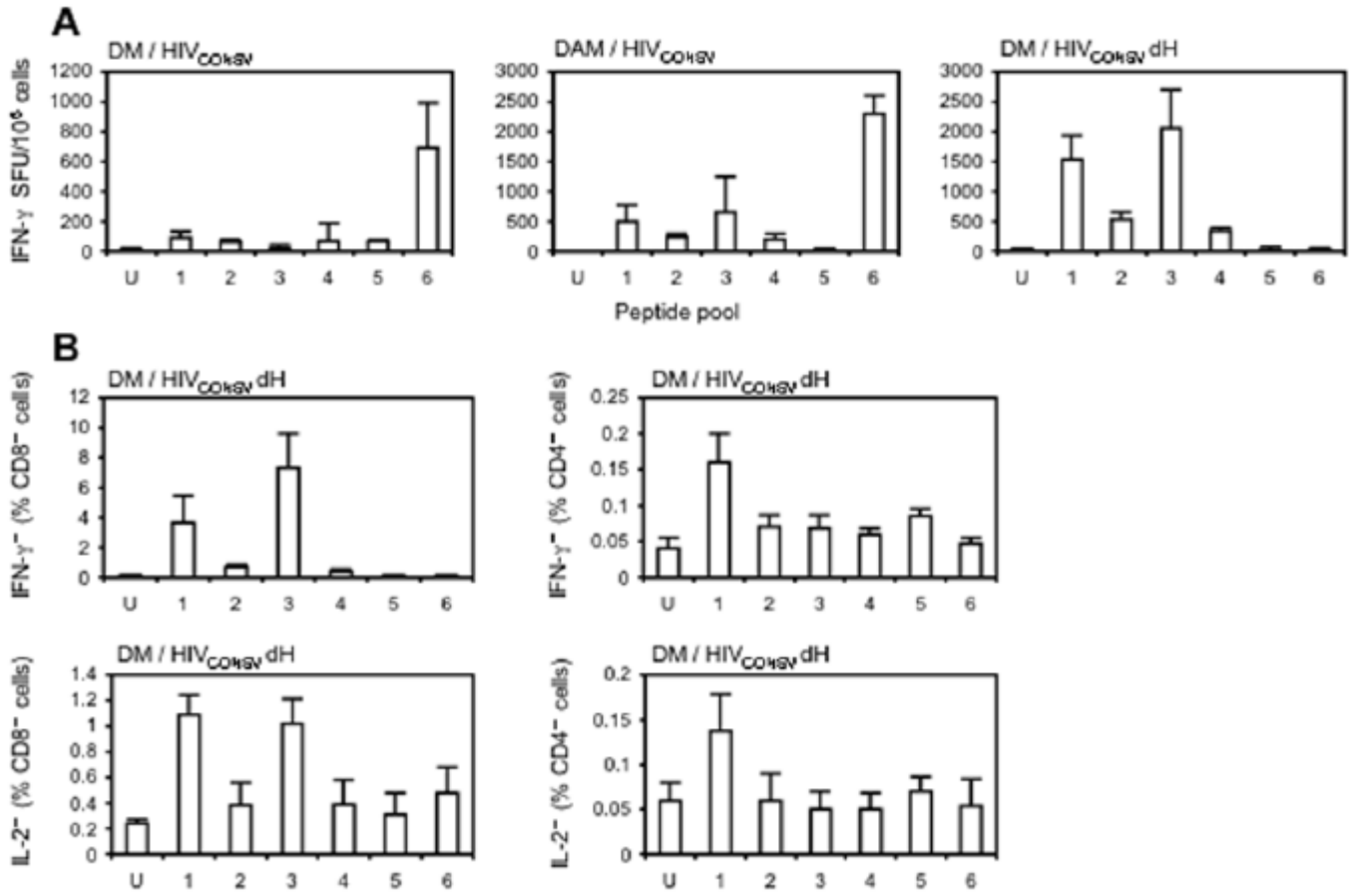
mAb C-terminal tag



Immunodominant epitope H

pTH DNA: pTH. HIV consv ——— D
Human adenovirus serotype 5(AdHu5): AdHu5.HIVconsv ——— A
Modified vaccinia virus Ankara(MVA) : MVA.HIVconsv ——— M

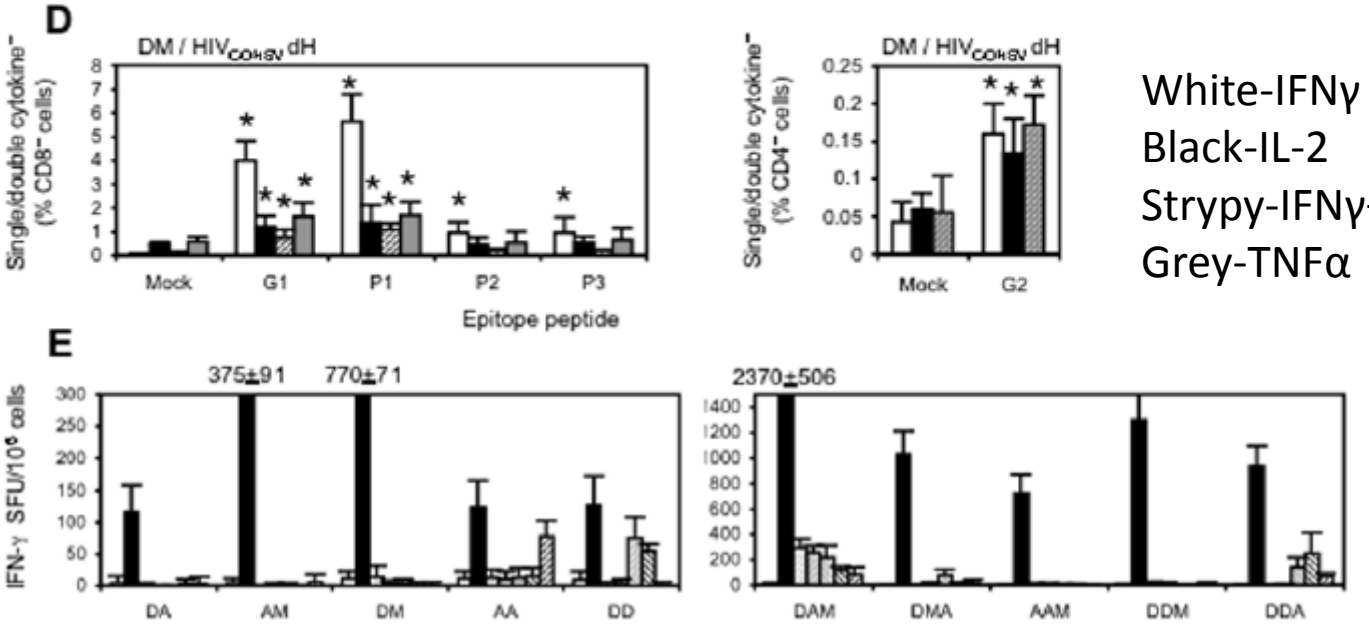
Breadth of HIVconsv vaccine induced immune responses in the BALB/c mice



Breadth of HIVconsv vaccine induced immune responses in the BALB/c mice

C

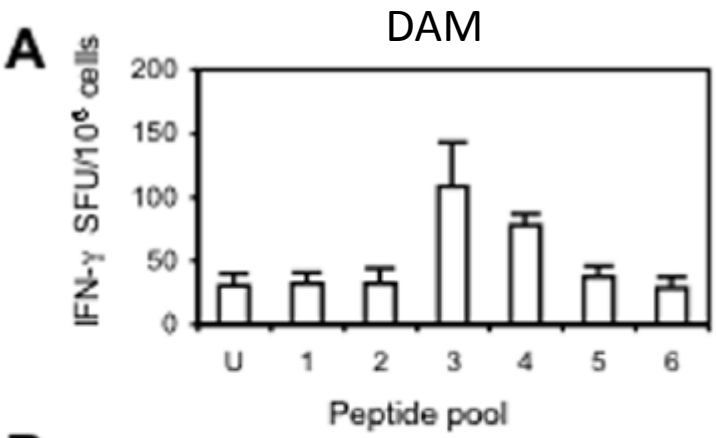
Peptide Pool No.	Peptide No.	HIV-1 Protein	Epitope/Peptide Sequence	Designation/ Reactivity
1	9, 10	Gag	AMQMLKDTI	G1 / CD8
1	15	Gag	IYKRWIILGLNKIVR	G2 / CD4
3	88, 89	Pol	IFQSSMTKI	P1 / CD8
4	111, 112	Pol	VYYDPSKDLI	P2 / CD8
2	42	Pol	KAIGTVLVGPTPVNI	P3 / CD8



White-IFNγ
Black-IL-2
Strypy-IFNγ+IL-2
Grey-TNFα

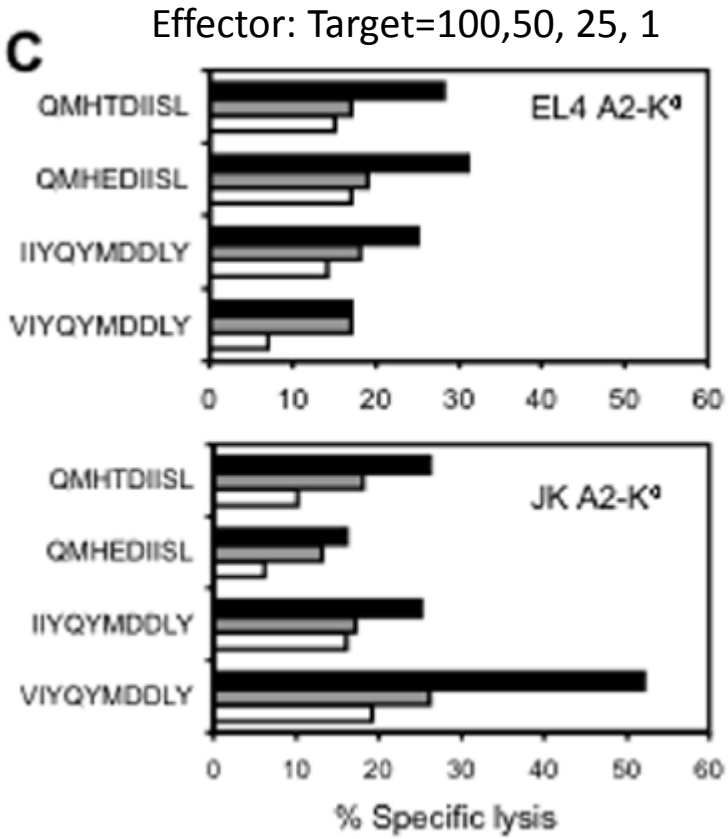
From left to right-no peptide, epitope H, G1, G2, P1, P2, P3.

HIVconsv vaccine induced HLA-A2-restricted responses in transgenic mice



B

Peptide Pool No.	Peptide No.	HIV-1 Gene	Epitope Sequence
3	94	Pol	VIYQYMDDLY
4	125	Env	QMHEDIISL



murine

^{51}Cr-release assay

human

Mice strain HHD, expresses MHC I molecule chimaeric human(α-1 and α-2) and mouse (α-3)*HLA-A*0201*heavy chain covalently linked to the human β2m light chain.
DAM regimen.

Generation of HIVconsv-specific responses in natural HIV-1 infection

Table 1. Tissue types and infecting viruses of human blood donors

Donor No.	HLA			HIV-1 Clade
	A*	B*	Cw*	
001[a]	1, 11	44, 51	7, 15	n.a.
002 [a]	2, 3	7, 13	6, 7	n.a.
003 [a]	1, 24	15, 18	3, 12	n.a.
004 [a]	1, 0201	7, 40	2, 7	n.a.
005 [a]	2, 3	7, 15	3, 7	n.a.
006 [a]	1, 0301	7, 08	7, 97	n.a.
007 [a]	2, 23/24	57, 42	2, 17	n.a.
008 [a]	1, 2	5001, 55	3, 6	n.a.
009 [a]	0201, 29	44, 13	6, 1601	n.a.
010 [b]	0102, 3303	44, 5802	n.d.	B/D
011 [b]	2, 29	45, 5802	6, 1601	D/A2 (CRF16)
012 [b]	2	15, 4402	3, 5	B
013 [b]	01, 11	18, 35	4, 7	B
014 [b]	24, 3401	40, 56	1, 4	B/C (CFR07)
015 [c]	3, 11	15, 4402	0303, 05	B
016 [c]	2601, 6802	70, 81	03, 04	n.d.
017 [c]	24	7, 18	7, 16	n.d.
018 [c]	1, 6801	5001, 1517	6, 7	n.d.
019 [c]	2601, 6802	70, 81	3, 4	n.d.
020 [c]	29, 32	7, 4401	1601	n.d.
021 [c]	0201, 0205	7, 18	n.d.	n.d.
022 [c]	03, 11	4201, 5301	4, 17	C

HIV-

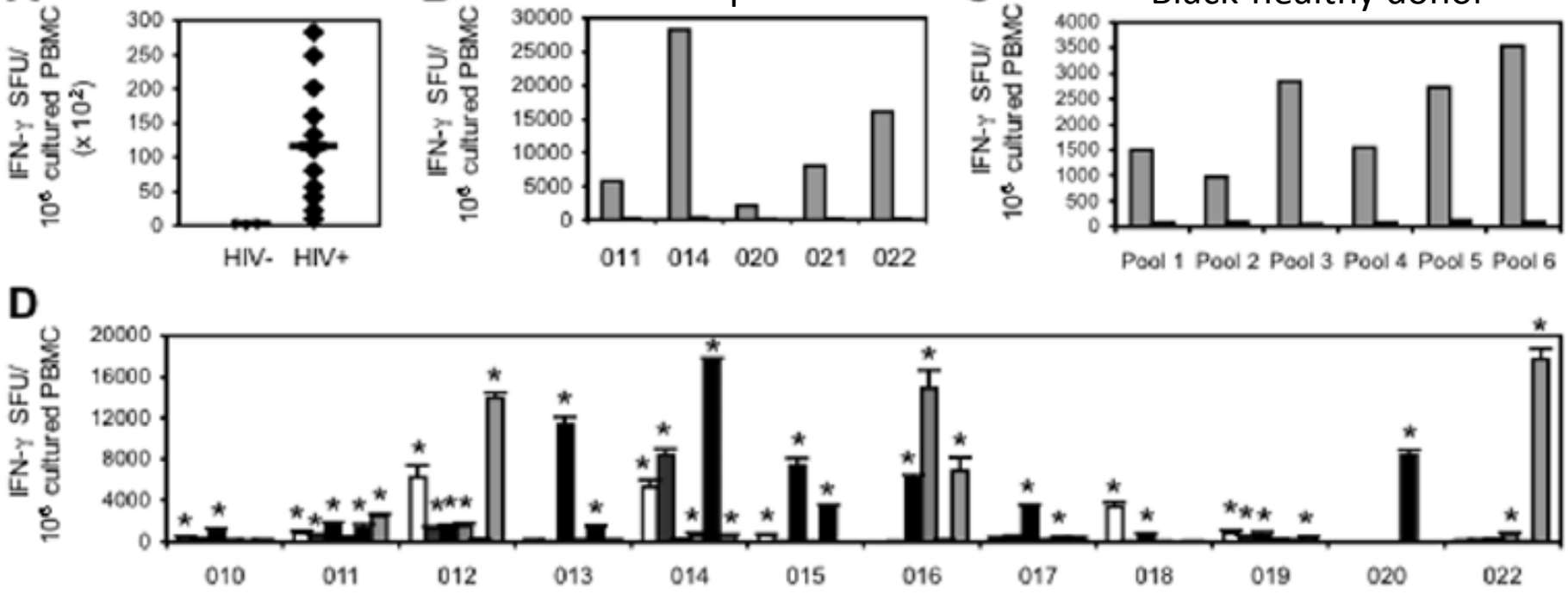
HIV+

[a]HIV-1/2-uninfected subjects
[b]UK HIV-1-infected patients vaccinated with HIVA vaccines
[c]Patients infected with HIV-1 in Africa [4]
n.a. – not applicable; n.d. not done
doi:10.1371/journal.pone.0000984.t001

Recognition of HIVconsv-derived peptides by PBMC from HIV-1 infected patient

Grey-CD8+ undepleted PBMC
Black-CD8+ depleted PBMC

Grey-HIV-1 infected
Black-healthy donor



Summary

- The HIVconsv immunogen is a chimaeric protein assembled from protein regions rather than epitopes, enable broader coverage.
- Consensus sequences allow a geographically broad deployment of the vaccine.
- Artificial clade consensus sequence designed deal with intra-clade variability.
- Combines sequences of 4 clades sequentially to avoids epitope antagonism.
- With lower frequency of immunodominant epitopes, induce broader T cell responses.
- Have to further identify the influence of the junction regions.

European Journal of
Immunology

Long peptides induce polyfunctional T cells against conserved regions of HIV-1 with superior breadth to single-gene vaccines in macaques

Maximillian Rosario[1], Anne Bridgeman[1], Esther D. Quakkelaar[2], Maire F. Quigley[3], Brenna J. Hill[3], Maria L. Knudsen[4], Virginia Ammendola[5], Karl Ljungberg[4], Nicola Borthwick[1], Eung-Jun Im[1], Andrew J. McMichael[1], Jan W. Drijfhout[2], Hui Yee Greenaway[6], Vanessa Venturi[6], Daniel C. Douek[3], Stefano Colloca[5], Peter Liljeström[4], Alfredo Nicosia[5], David A. Price[3,7], Cornelis J. M. Melief[2] and Tomáš Hanke[1]

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EFFICACY IN ANIMAL MODELS

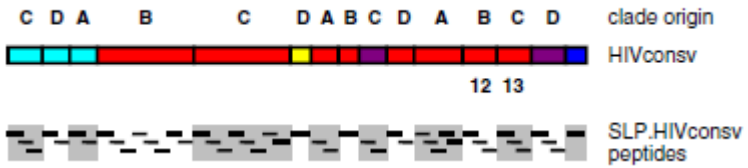
Main message:

- Vaccine tested: pTH. HIV consv, AdHu5.HIVconsv, MVA.HIVconsv, SLP.HIVconsv , ChAdV63.HIVconsv , VREP.HIVconsv

- Immunophenotype and clonal composition of CM9-specific CD8+ T-cell populations

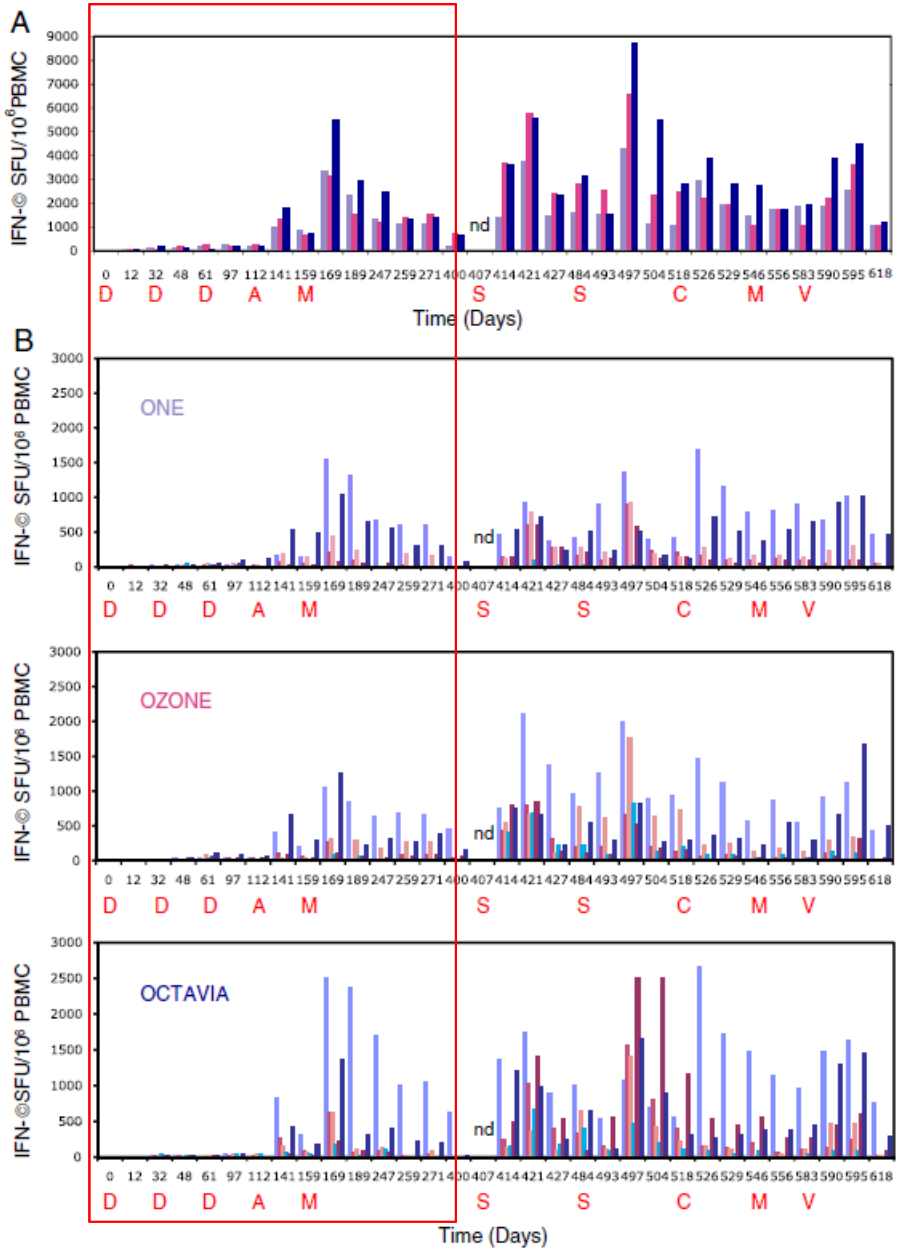
- Test immunogenicity in macaques

DDDAM regimen induces high frequencies of HIV-1 spcific T cells responses in macaques

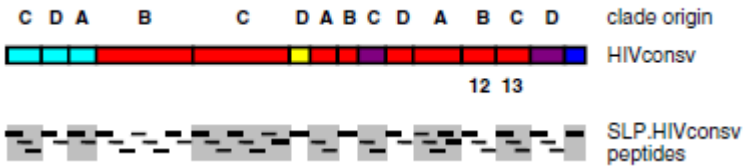


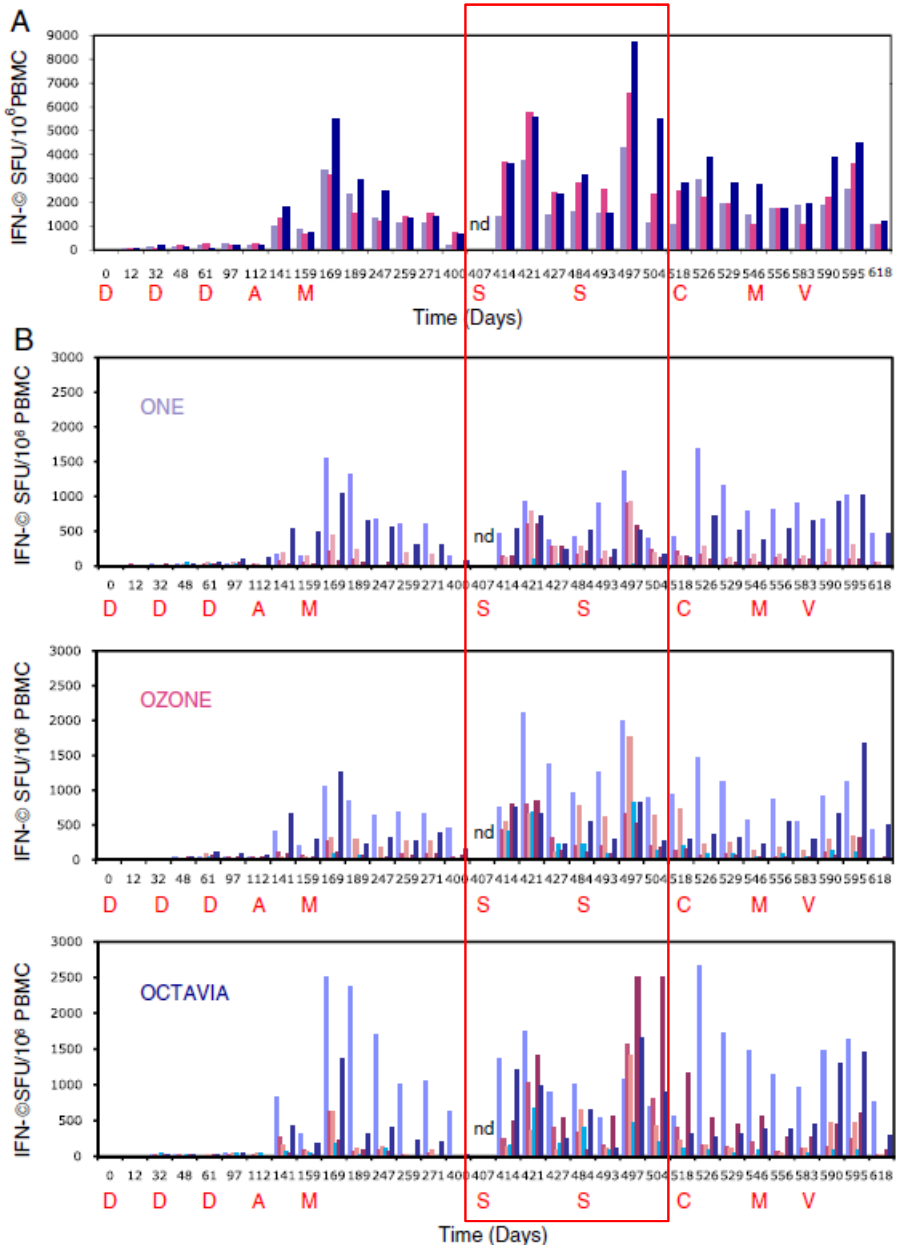
Cyan-gag
Red-pol
Yellow-vif
Purple-env
Blue-**CM9 epitope**-rhesus macaque Mamu-A*01-restricted CD8+T cells
+BALB/c mouse CD8+T cell epitope
+mAB tag PK

DDDAM-intramuscularly



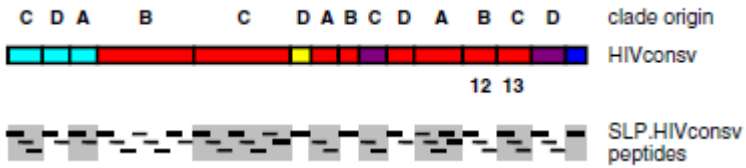
Adjuvanted SLP elicit T-cell responses superior epitope breadth compared with sORF genetic vaccines

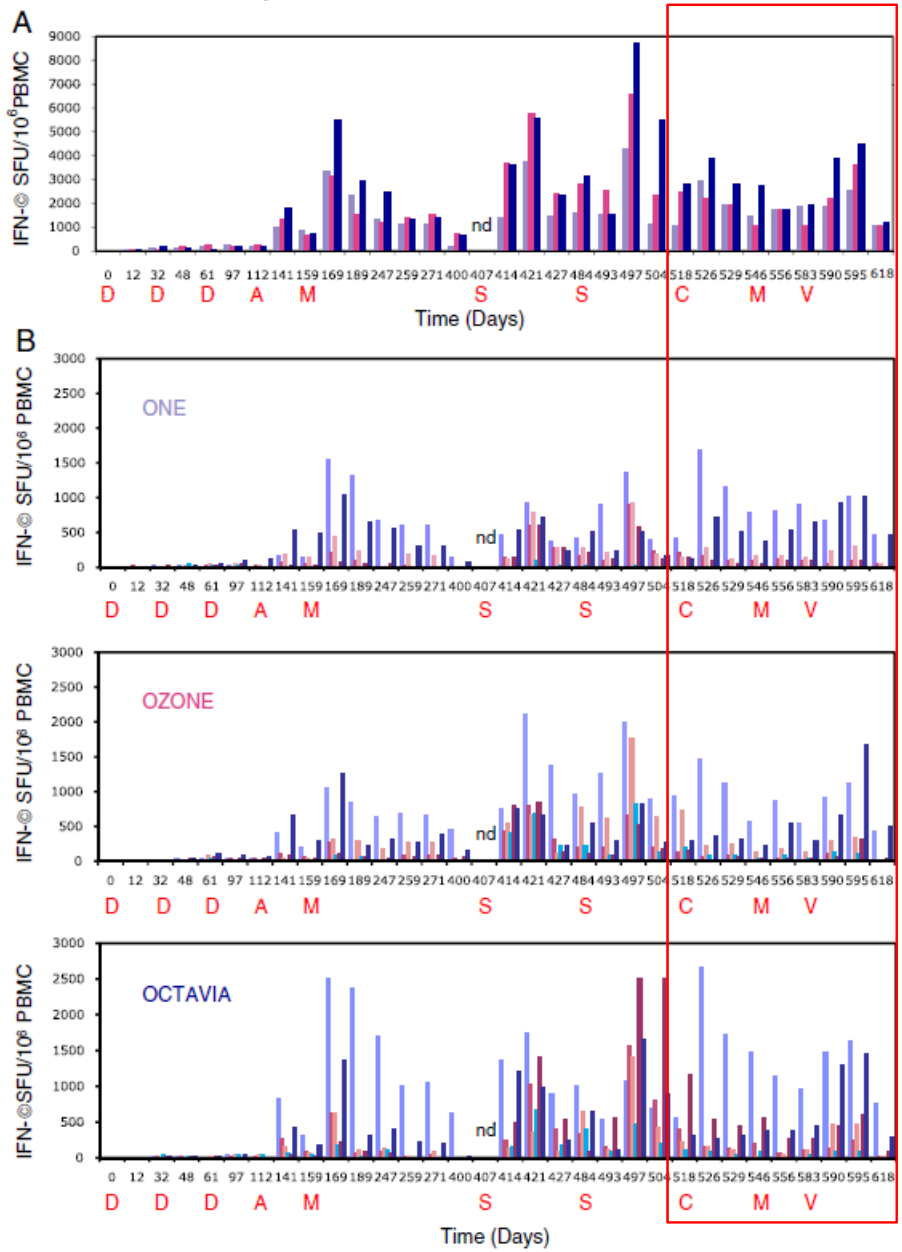




S: 46 peptides, 25-28 amino acids long with 10-14 residue overlap, emulsified in Montanide ISA-51, subcutaneously inject into 2xarm, 2xback, 2xthigh

sORF genetic vaccines do not maintain the breadth of SLP-induced responses





C: ChAdV63.HIVconsv, use simian adenovirus 63 vector
V: VREP.HIVconsv, use Semliki Forest virus vecotor

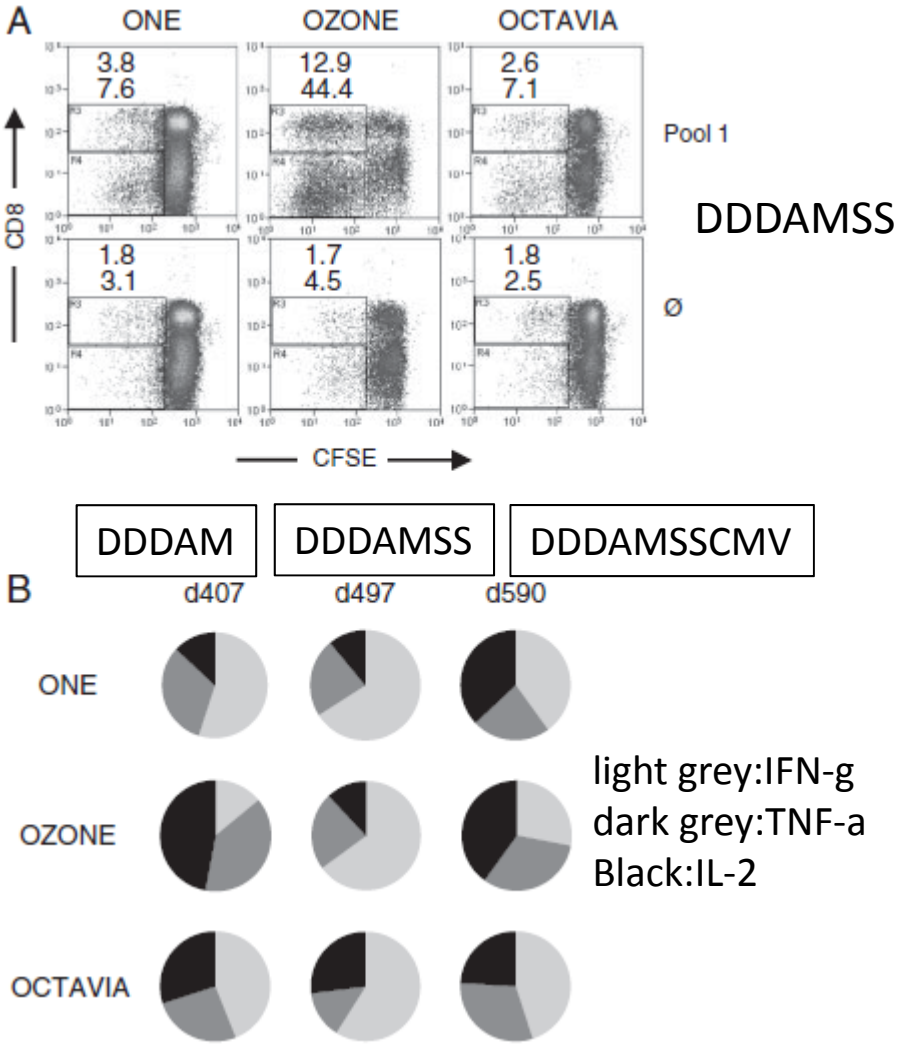
SLP.HIVconsv induces responses to multiple CD8+ and CD4+ T cell epitopes

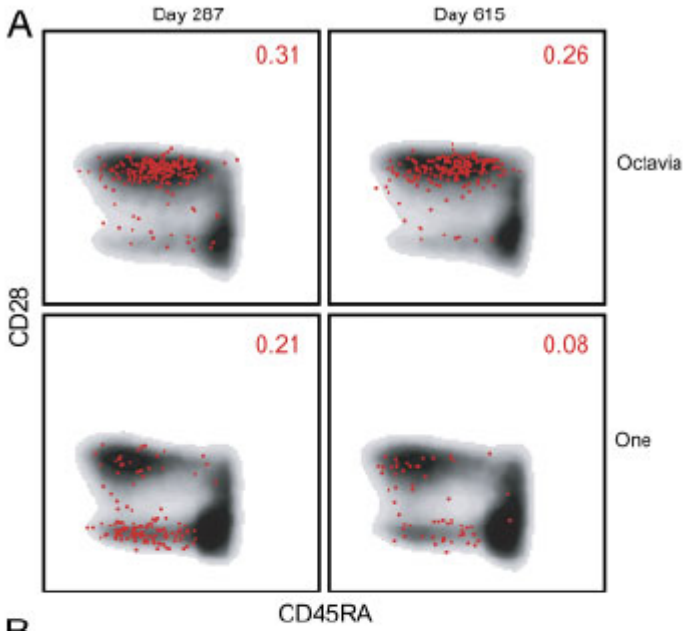
Table 1. Peptides and/or epitopes recognized by CD8$^+$ T cells in HIVconsv-vaccinated rhesus macaques

HIVconsv peptide no.	HIV origin	Peptide sequence	ONE	OZONE	CTAVIA
(1, 2)[a]	Gag	FSPEVIPMF	+[b]	+	+
14	Gag	EWDR-IYKRWIILGLN[c]	–	+	–
15	Gag	IYKRWIILGLNKIVR	+	+	–
16	Gag	WIILGLNKIVRMYSP	–	+	–
17	Gag	GLNKIVRMYSPVSIL	+	+	+
(18,19)[a]	Gag	YSPVSILDI	+	+	–
36	Pol	MIGGIGGFIKVRQYD	+	+	+
37	Pol	IKVRQYDQILIEICG**KK**[d]	–	+	+
45	Pol	VNIIGRNLLTQIGCT**KK**[d]	+	–	–
88	Pol	GSPAIFQSSMTKILE	+	–	–
89	Pol	IFQSSMTKILEPFRA	+	–	–
93	Pol	KNPEIVIYQYMDDLYV	–	+	–
97	Pol-Vif	SDLEIGQHR-MENRWQ[c]	+	+	–
146	Pol	SPGIWQLDCTHLEGK	–	–	+
160	Pol	IIGQVRDQAEHLKTA	–	–	+
161	Pol	VRDQAEHLKTAVQMA	–	–	+
(162, 163)[a]	Pol	KTAVQMAVF	–	+	+
164	Pol	VQMAVFIHNFKRKGGI	–	–	+
(167, 168)[a]	Pol	YSAGERI-WK[c]	+	+	–
173	Pol	GAVVIQDNSDIKVVP	–	–	+
(175, 176)[a]	Pol	VVPRRKAKI	+	–	–
(182, 183)[a]	Env	GSTNGAASMTL	+	+	+
(194, 195)[a]	SIV Gag CM9	CTPYDINQML	+	+	+
198[e]	Env-SV5	RGPGRAFVTIPNPLL	–	–	+
199[e]	Env-SV5	RAFVTIPNPLLGLD	–	–	+

[a] Sequence next to two peptides in brackets indicates that an optimal epitope contained within those peptides was used.
[b] A positive response means that specific IFN-γ release to peptide stimulation was detected on at least two separate occasions.
[c] Junction between two adjacent regions in HIVconsv is shown by dash.
[d] Peptides 37 and 45 were modified by the addition of two lysines at the C-terminus to increase solubility.
[e] Peptides 198 and 199 are not derived from the conserved HIV-1 regions, but rather from a BALB/c mouse T-cell epitope in HIV-1 Env and mAb tag originating from simian virus 5, which were added to the HIVconsv immunogen to facilitate preclinical development.

HIVconsv vaccines induce polyfunctional, oligoclonal T-cell populations





B

ID	Day 287 TRBV	CDR3	TRBJ	Freq.	Day 615 TRBV	CDR3	TRBJ	Freq.
Octavia	5-6	CASTKGGTRTDPQY	2-3	85	6-1	CASSDQGNSNQPQY	1-5	46
	5-1	CASSLDRETAQLF	2-2	7	7-4	CASSLTGNSNSPLY	1-6	14
	6-3	CASTPGTGGTKNTQY	2-4	3	5-6	CASTEGGTRTDPQY	2-3	10
	6-2	CASSGEGNSNQPQY	1-5	3	11-1	CASRPTPSSGNTVY	1-3	9
	5-6	CASTKGETRTDPQY	2-3	1	5-1	CASSLDRETAQLF	2-2	5
	6-3	CASTPGTGRTKNTQY	2-4	1	6-2	CASSEGGNSNQPQY	1-5	5
					11-1	CASSLDSNQNTQY	2-4	3
					4-2	CASSLAQGRRTEAF	1-1	3
					6-2	CASSEASRGADPQY	2-3	3
					6-1	CASSDQGNSNRPQY	1-5	2
One	6-1	CASSEAYHSTDPQY	2-3	64	6-3	CASRACGSSANQLF	2-2	32
	3-1	CASSQGPSNSNQPQY	1-5	21	3-1	CASSQGPSNSNQPQY	1-5	32
	7-2	CASSPSGSIDYT	1-2	12	21-1	CASSISEFYNSPLY	1-6	21
	5-6	CASSSGTGGSRAQLF	2-2	1	6-2	CASSERTGSQNTQY	2-4	10
	6-1	CASREAYHSTDPQY	2-3	1	21-1	CASSISELYNSPLY	1-6	5
	6-1	CASSEAYHSTDPQY	2-3	1				

Summary

- The DDDAM regimen, which mimics one of the planned clinical schedules, was highly immunogenic in macaques .
- Adjuvanted SLP.HIVconsv, partitioned and delivered at disparate anatomical sites, elicited broader and more potent T-cell responses that were neither induced nor maintained by single- ORF genetic vaccines .
- A ‘‘ceiling’’ effect appeared to operate for certain specificities with repeated vaccinations in a complex heterologous regimen, such that response magnitude was not enhanced substantially beyond a certain vaccination.
- The extent to which T cells elicited in response to HIVconsv vaccines actually recognize and kill HIV-1-infected human cells remains to be determined.
- The animals in this study cannot be challenged.

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Superior Induction of T Cell Responses to Conserved HIV-1 Regions by Electroporated Alphavirus Replicon DNA Compared to That with Conventional Plasmid DNA Vaccine

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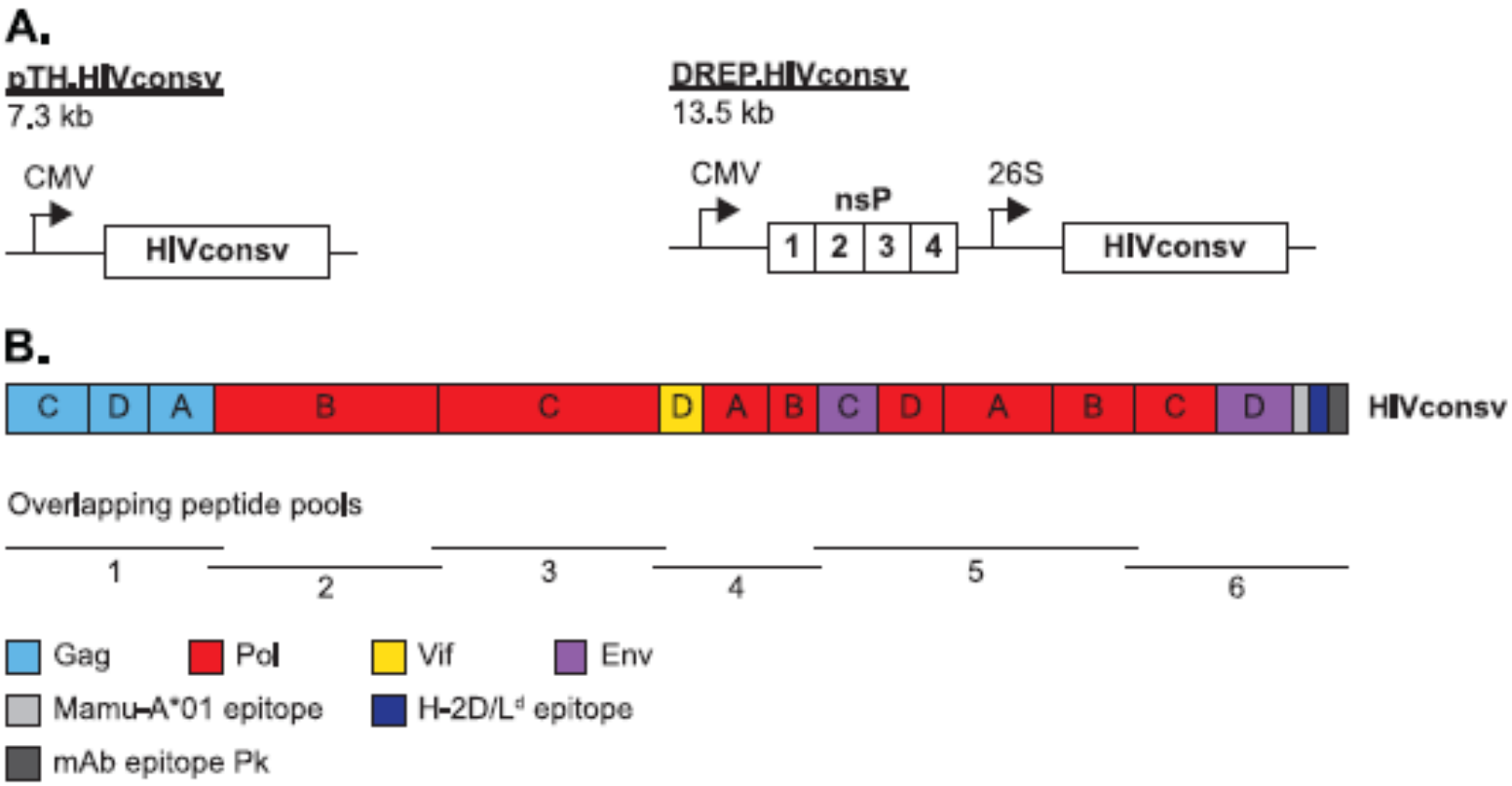
Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden[a]; The Jenner Institute, University of Oxford, Oxford, United Kingdom[b]; MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom[c]; Okairòs, Rome, Italy[d]; CEINGE, Naples, Italy[e]; and Department of Biotechnology, University of Naples, Naples, Italy[f]

EFFICACY IN ANIMAL MODELS

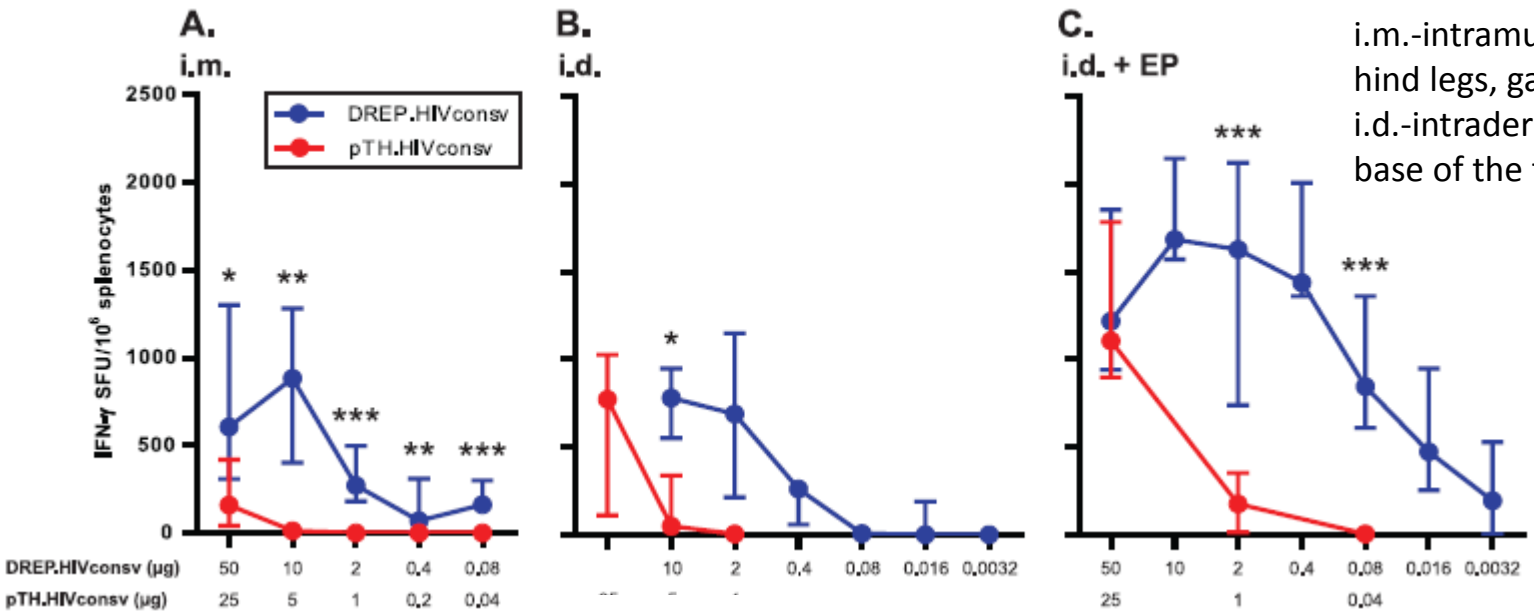
Main message:

- Vaccine tested: pTH. HIV consv, DREP.HIVconsv , MVA.HIVconsv, ChAdV63.HIVconsv
- Specific CD8+ T-cell responses
- Test immunogenicity in mice and macaques

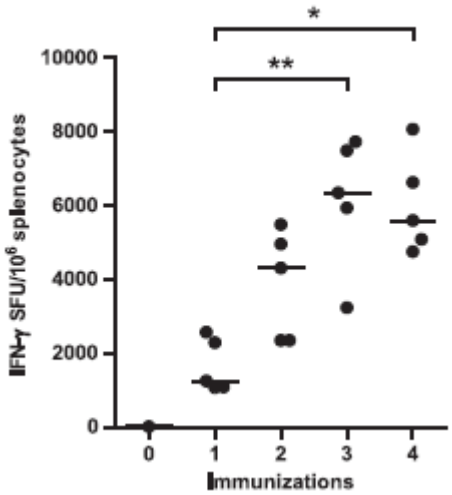
DNA constructs and HIVconsv immunogen



Much lower doses of DREP.HIVconsv than plasmid pTH.HIVconsv DNA are required for indution of CD8+ T cell responses



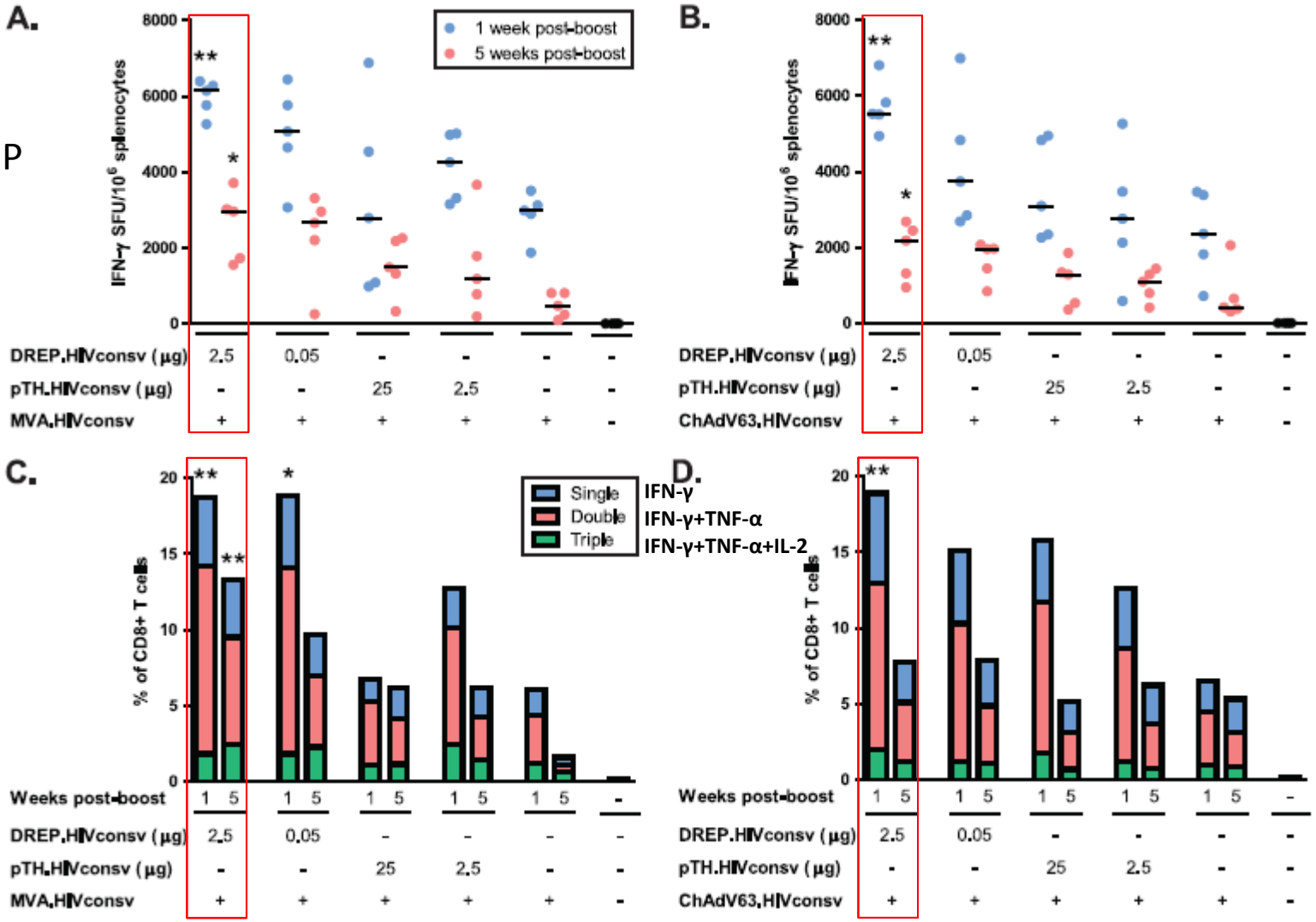
i.m.-intramuscular
hind legs, gastrocnemius muscle
i.d.-intradermal
base of the tail



DREP.HIVconsv, 2.5ug, EP administered monthly

The DREP.HIVconsv vaccine potently primes CD8+ T cells prior to a heterologous boost immunization

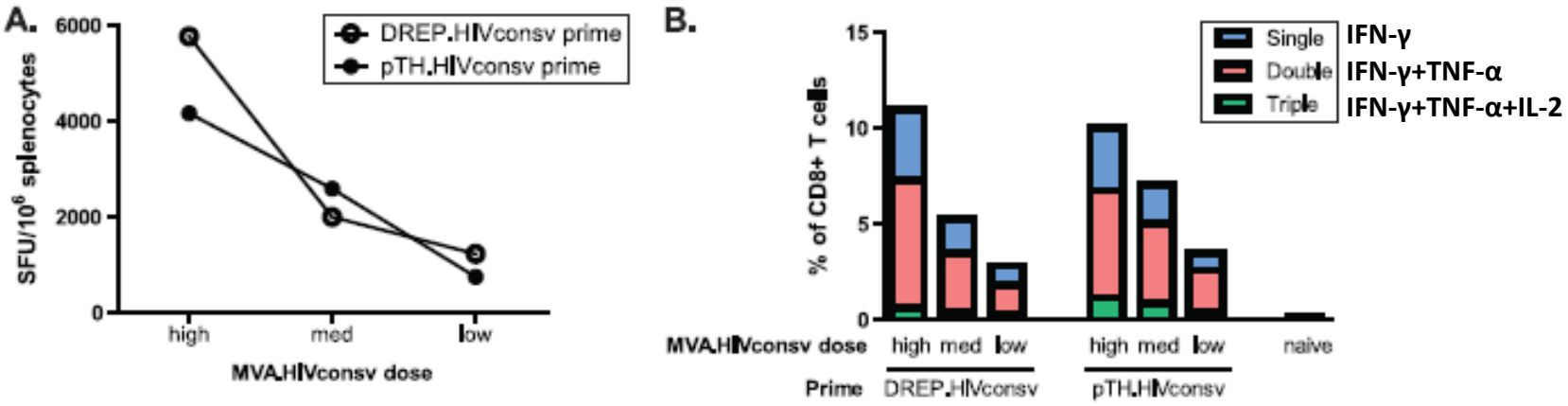
i.d. EP



5x106 PFU MVA. HIVconsv

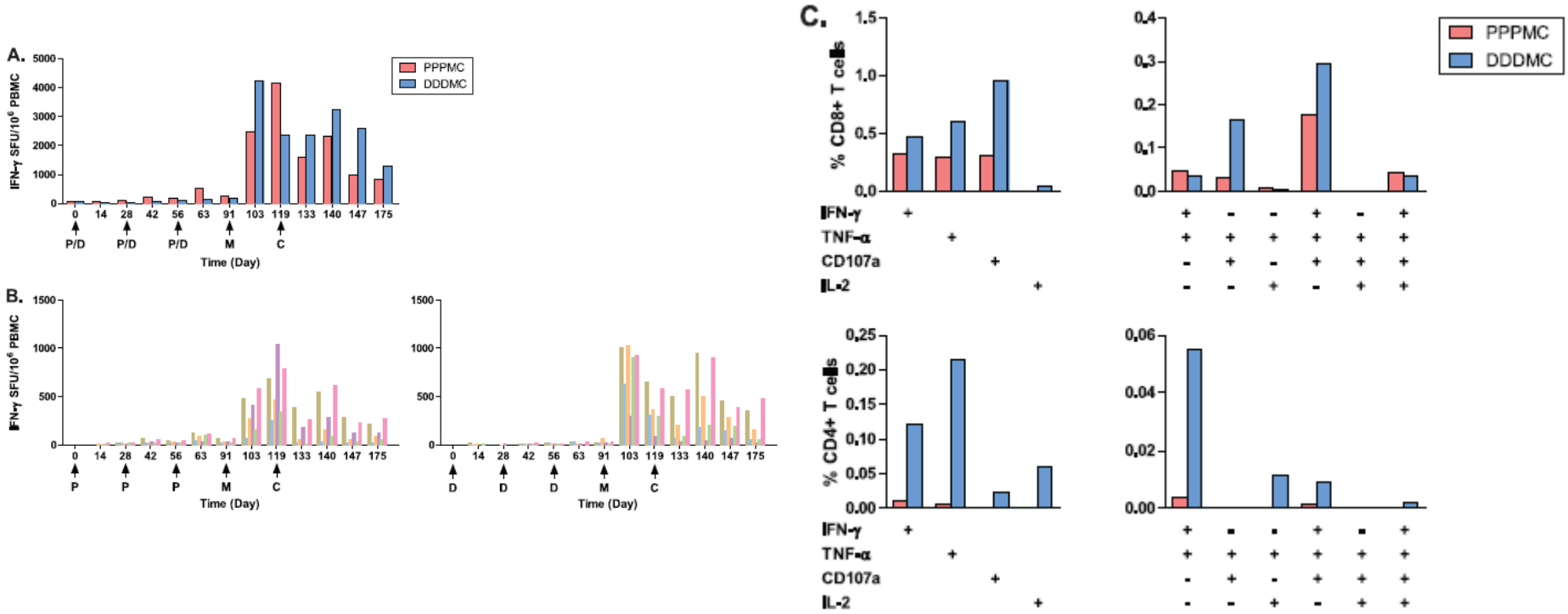
7x109 vp ChAdV63. HIVconsv

MVA.HIVconsv dose test



DREP.HIVconsv-50ng
pTH.HIVconsv-2.5ug
4 weeks later, boost with5x106 (high), 5x105 (medium), 5x104 (low) PFU MVA. HIVconsv

A 20-fold-lower dose of DREP.HIVconsv than of plasmid DNA induces an equivalent response in rhesus macaques



P: pTH.HIVconsv, 4mg, i.m.
D:DREP.HIVconsv, 400ug, i.m.
M: MVA. HIVconsv, 108PFU, i.m.
C: ChAdV63. HIVconsv, 1010 vp, i.m.

Summary

- Immunogenicity of DNA vaccines can be strongly enhanced by the use of an alphavirus replicon DNA vector (125 times lower dose) and EP (625 times lower dose).

- T cell responses primed by replicon DNA can be further boosted by recombinant MVA- and attenuated chimpanzee adenovirus-vectored vaccines.

- Does titration in macaques is necessary.

Prime-boost regimens with adjuvanted synthetic long peptides elicit T cells and antibodies to conserved regions of HIV-1 in macaques

Maximillian Rosario[a], Nicola Borthwick[a,b], Guillaume B. Stewart-Jones[a], Alice Mbewe-Mvula[a,b], Anne Bridgeman[a,b], Stefano Colloca[c], David Montefiori[d], Andrew J. McMichael[a], Alfredo Nicosia[c,e], Esther D. Quakkelaar[f], Jan W. Drijfhout[f], Cornelis J.M. Melief[f] and Tomáš Hanke[a,b]

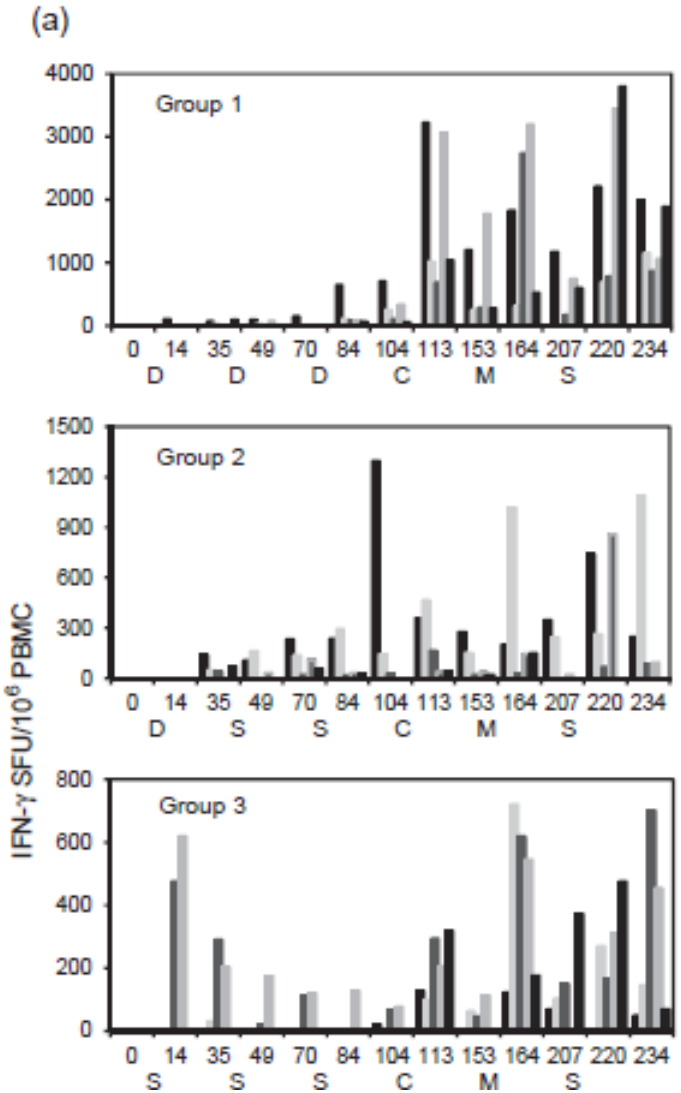
EFFICACY IN ANIMAL MODELS

- Vaccine tested: pSG2.HIVconsv DNA(D) ,imiquimod/montanide-adjuvanted SLP.HIVconsv (S), MVA.HIVconsv(M), ChAdV63.HIVconsv (C)

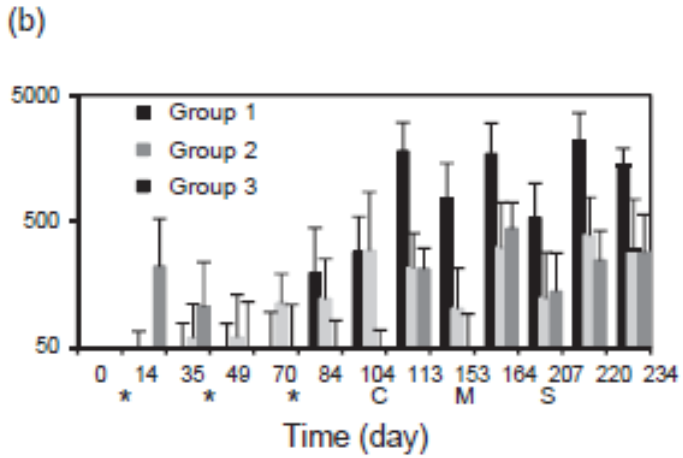
- Specific CD8+ T-cell responses

- Test Prime-boost regimens of DDDCMS, DSSCMS and SSSCMS in rhesus macaques.

DDD, but not DSS or SSS, primes consistently low T cell responses



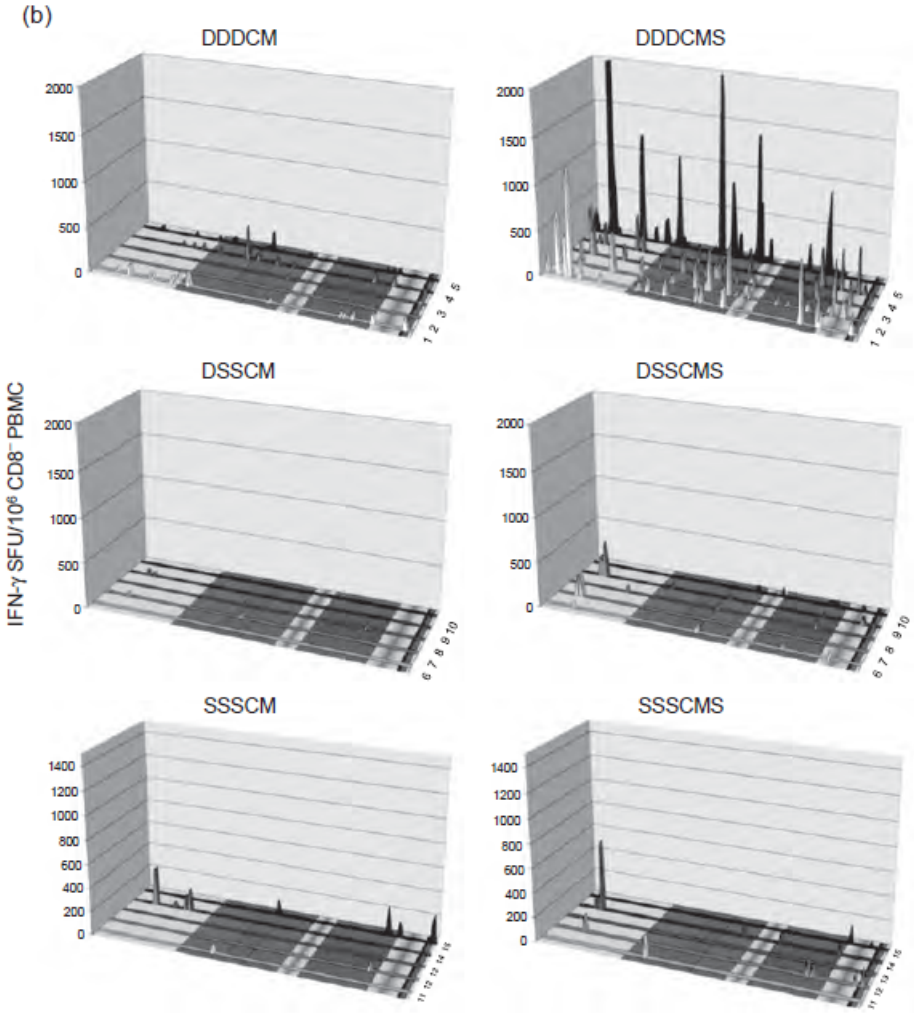
3 groups, 5 rhesus macaques each group



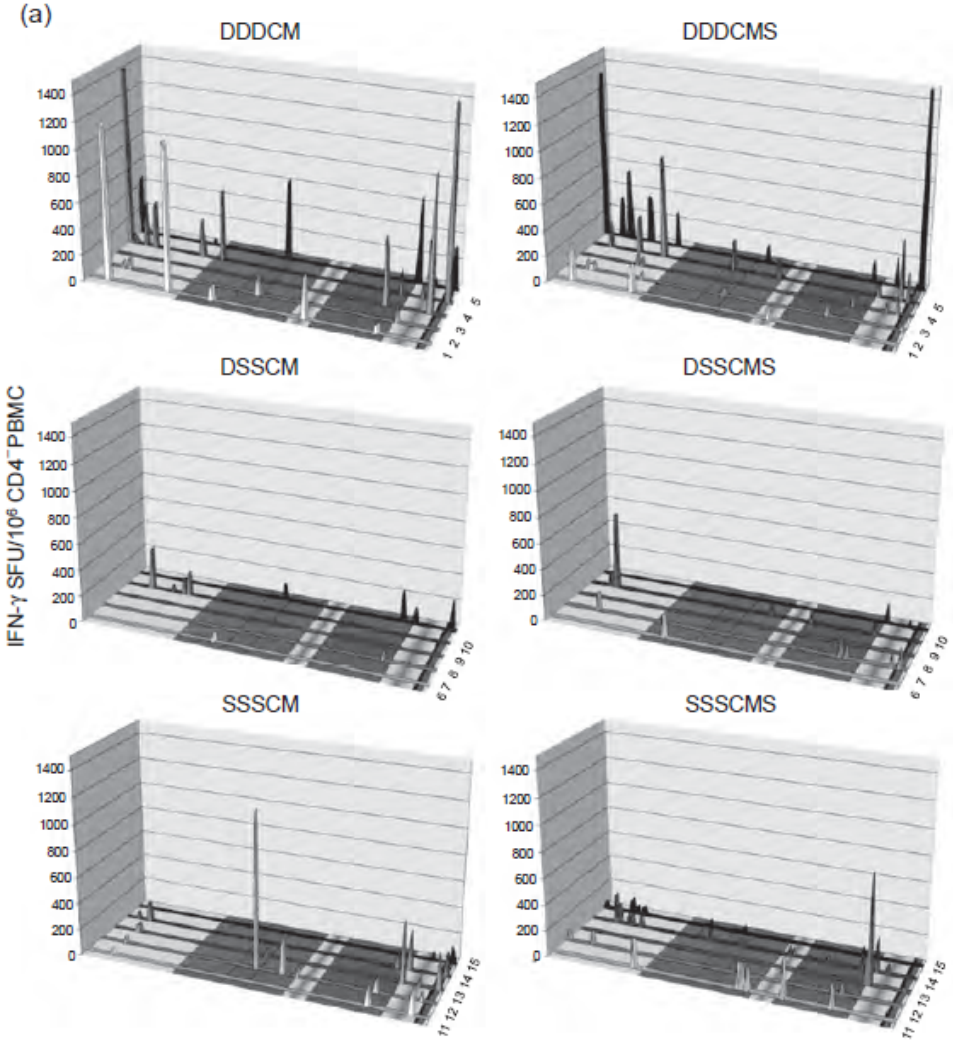
pSG2.HIVconsv DNA(D) , SLP.HIVconsv (S), MVA.HIVconsv(M), ChAdV63.HIVconsv (C)

Black-DDDCMS
Grey-DSSCMS
White-SSSCMS

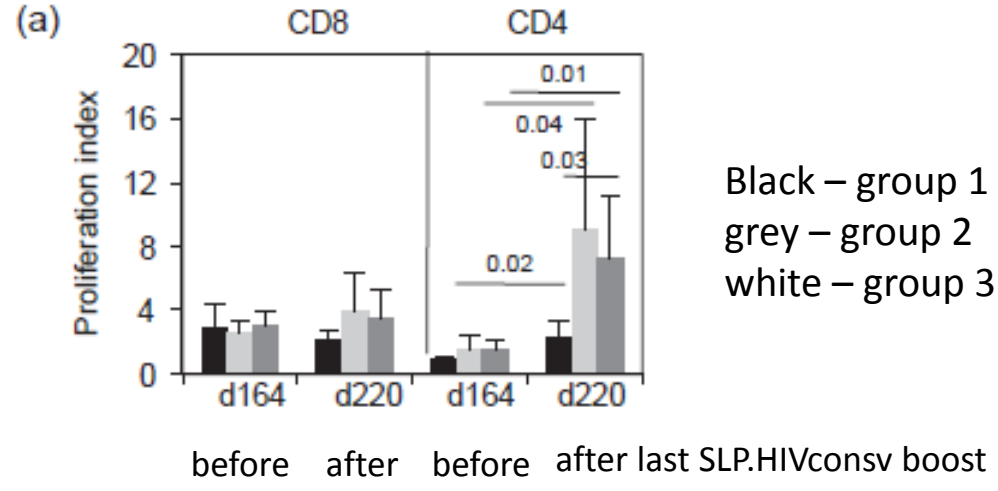
DDD primes for robust CD4+ T-cell expansions by CM, and SLP.HIVconsv boost broadens CD4+ T cell responses



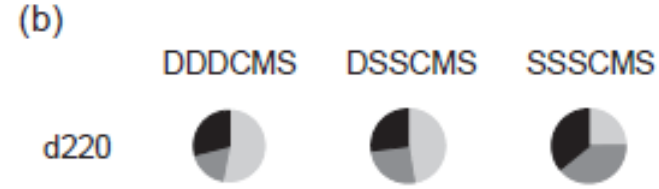
DDD primes for robust CD8+ T-cell expansions by CM



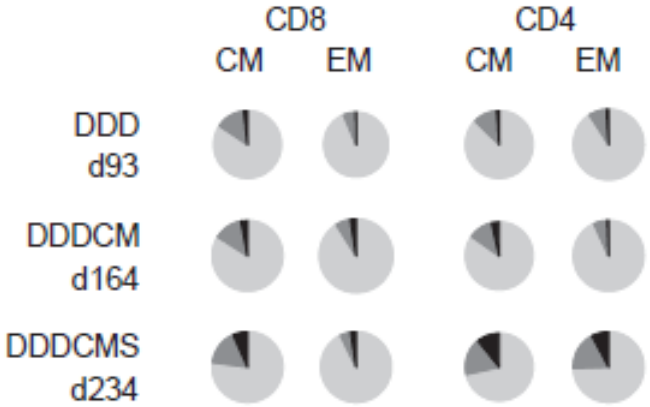
HIVconsv-specific T cells proliferate and are oligofunctional



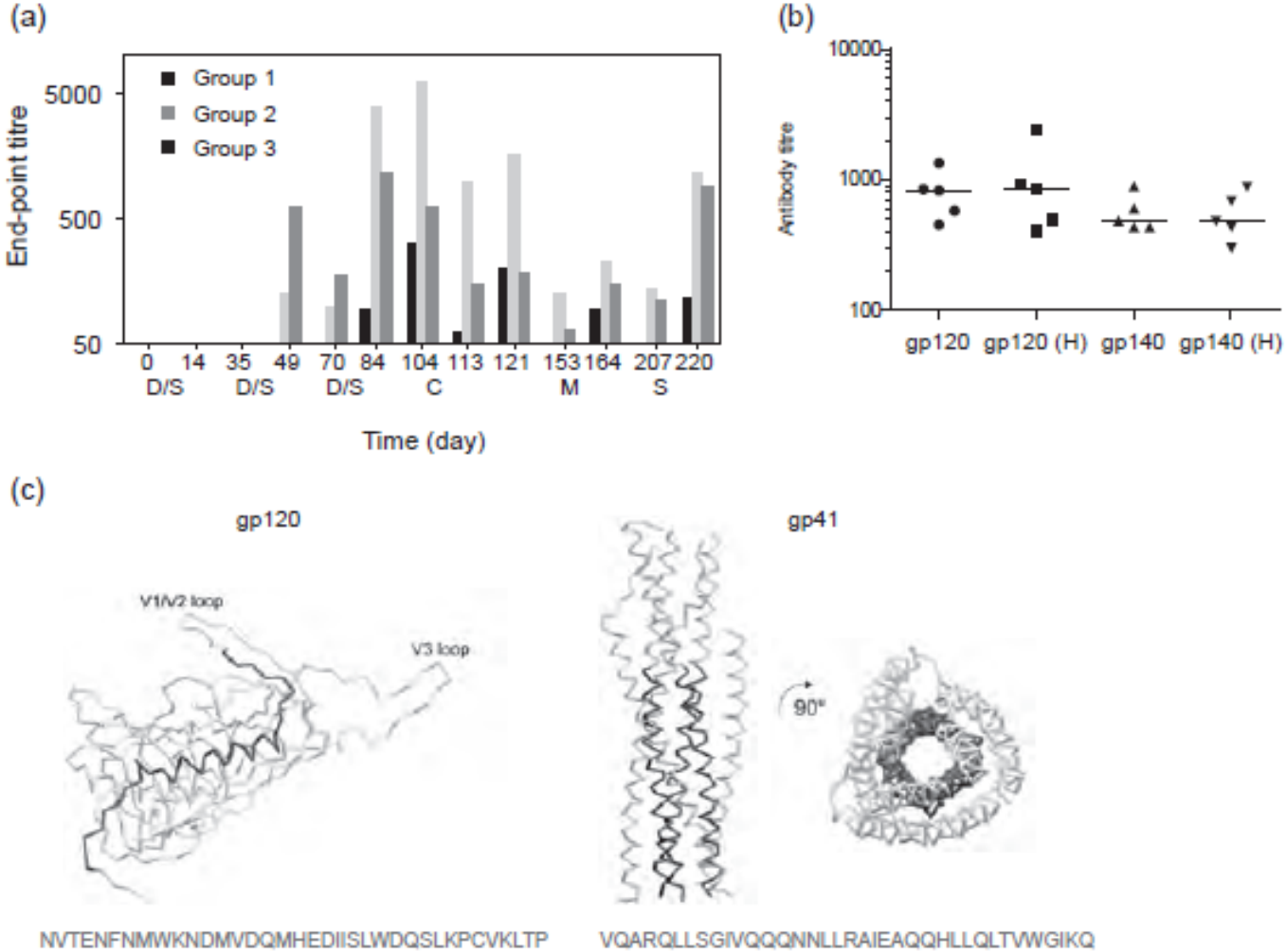
Black – group 1
grey – group 2
white – group 3



light grey-IFN-γ
dark grey- IFN-γ+TNF-α
black -IFN-γ+TNF-α+IL-2



SLP.HIVconsv induces antibodies to conserved regions of Env



Summary

- DDD primed for the largest subsequent expansions of HIV-1-specific T cells.
- DSS primed for the highest titres of Env-specific antibodies .
- Electroporation spares the DNA vaccine dose and SLP broadens the specificity of T cells induced by single-gene genetic vaccines.

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journal homepage: www.elsevier.com/locate/vaccine

Absence of systemic toxicity changes following intramuscular administration of novel pSG2.HIVconsv DNA, ChAdV63.HIVconsv and MVA.HIVconsv vaccines to BALB/c mice☆

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SAFETY IN ANIMAL MODELS

Table 1
Summary of animal treatments.

Group[a]	Treatment	Number of animals		Dosing			
		Male	Female	Day 1	Day 15	Day 29	Day 43
1	Control	10	10	PBS	PBS	PBS	PBS
2	DDDC	10	10	50 µg pSG2.HIVconsv[b]	50 µg pSG2.HIVconsv	50 µg pSG2.HIVconsv	5.95×10^9 vp ChAdV63.HIVconsv[b]
3	Control	10	10	PBS	PBS	PBS	ND
4	MMM	10	10	2×10^7 pfu MVA.HIVconsv[c]	2×10^7 pfu MVA.HIVconsv	2×10^7 pfu MVA.HIVconsv	ND

ND – not dosed.

[a] This summarised two studies UNO012 and UNO011 testing toxicity of DDDC and MMM regimens, respectively.

[b] Injected volumes i.m. of the pSG2.HIVconsv DNA and ChAdV63.HIVconsv vaccines were 50 µl.

[c] Injected volume i.m. of the MVA.HIVconsv vaccine was 20 µl.

Table 2
Tissues examined at necropsy, weighed and subjected to histopathological examination.

Macroscopic abnormalities	Parenteral sites (right hind limb, muscle and overlying skin)
Adrenals	Peyer's patches
Aorta – thoracic	Pituitary
Brain	Prostate
Caecum	Rectum
Carcass	Salivary glands
Colon	-Submandibular[a,b]
Duodenum	-Parotid[a]
Epididymides	-Sublingual[a,b]
Eyes	Sciatic nerves[a]
Femurs[a]	Seminal vesicles[b]
Gall bladder	Skeletal muscle[a]
Harderian glands	Skin with mammary glands
Heart	Spinal cord
Ileum	Spleen[b]
Jejunum	Sternum
Kidneys[b]	Stomach
Lachrymal glands	Testes[b]
Larynx	Thymus[b]
Liver[b]	Thyroid with parathyroids
Lungs[b]	Tongue
Lymph nodes	Trachea
-Mandibular	Ureters
-Popliteal	Urinary bladder
Oesophagus	Uterus and cervix[b]
Optic nerves	Vagina
Ovaries[b]	
Pancreas	

[a] Only one processed for examination.
[b] Weighed (salivary glands weighed together, lung weight included mainstem bronchi).

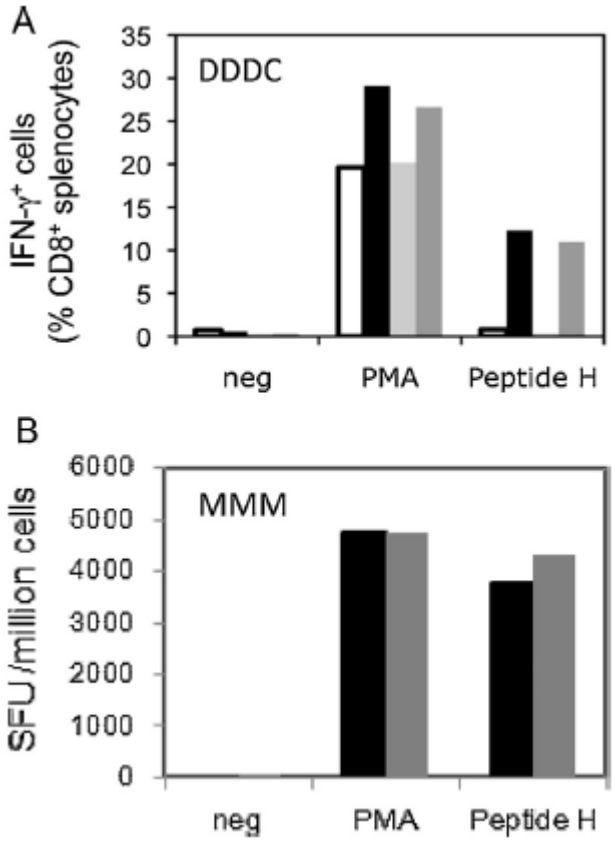
A
DDDC
IFN-γ+ cells
(% CD8+ splenocytes)
35
30
25
20
15
10
5
0
neg
PMA
Peptide H
B
MMM
SFU/million cells
6000
5000
4000
3000
2000
1000
0
neg
PMA
Peptide H

Table 3a
Haematology for DDDC.[a]

Group/sex[b]		Hct (l/l)	Hb (g/dl)	RBC ($\times 10^{12}$/l)	Retic (%)	MCH (pg)	MCHC (g/dl)	MCV (fl)	WBC ($\times 10^{9}$/l)	N ($\times 10^{9}$/l)	L ($\times 10^{9}$/l)	E ($\times 10^{9}$/l)	B ($\times 10^{9}$/l)	M ($\times 10^{9}$/l)	LUC ($\times 10^{9}$/l)	Plt ($\times 10^{9}$/l)
1M	Mean	0.518	16.0	10.69	2.21	15.0	30.8	48.4	4.48	0.87	3.37	0.12	0.01	0.09	0.03	1100
	SD	0.0073	0.25	0.159	0.115	0.15	0.17	0.49	1.667	0.295	1.297	0.041	0.005	0.033	0.026	48.5
2M	Mean[c]	0.512	15.9	10.67	2.28	14.9	31.0	48.1	6.80**	1.20*	5.28**	0.13	0.01*	0.13*	0.05	1198**
	SD	0.0097	0.23	0.210	0.144	0.31	0.42	0.80	1.172	0.223	1.108	0.051	0.005	0.042	0.012	65.0
1F	Mean	0.492	15.3	9.87	2.26	15.5	31.2	49.8	2.89	0.54	2.21	0.07	0.00	0.05	0.02	1018
	SD	0.0090	0.33	0.217	0.344	0.14	0.46	0.83	1.522	0.332	1.181	0.033	0.005	0.021	0.013	23.2
2F	Mean[c]	0.509*	16.0*	10.46**	2.88*	15.3*	31.4	48.7**	4.00	0.85	2.94	0.09	0.01	0.09	0.02	1119**
	SD	0.0167	0.64	0.387	0.683	0.17	0.68	0.86	2.407	0.545	1.796	0.053	0.005	0.057	0.021	90.3

Hct – haematocrit; Hb – haemoglobin; RBC – erythrocyte; Retic – reticulocyte; MCHC – mean cell haemoglobin concentration; MCV – mean cell volume; WBC – total white cell count; N – neutrophils; L – lymphocytes; E – eosinophils; B – basophils; M – monocytes; LUC – large unstained cells; Plt – platelet.

[a] Animals received 3 doses i.m. of pSG2.HIVcosv DNA followed by 1 dose i.m. of ChAdV63.HIVconsv.

[b] 1M – males control; 2M – males receiving DDDC; 1F – females control; 2F – females receiving DDDC.

[c] *t*-Test: $^{*}p < 0.05$, $^{**}p < 0.01$.

Table 3b
Haematology for MMM.[a]

Group/sex[b]		Hct (l/l)	Hb (g/dl)	RBC ($\times 10^{12}$/l)	Retic (%)	MCH (pg)	MCHC (g/dl)	MCV (fl)	WBC ($\times 10^{9}$/l)	N ($\times 10^{9}$/l)	L ($\times 10^{9}$/l)	E ($\times 10^{9}$/l)	B ($\times 10^{9}$/l)	M ($\times 10^{9}$/l)	LUC ($\times 10^{9}$/l)	Plt ($\times 10^{9}$/l)
1M	Mean	0.466	15.8	10.62	2.59	14.8	33.8	43.9	1112	6.90	0.98	5.61	0.10	0.01	0.15	0.05
	SD	0.0143	0.43	0.318	0.192	0.21	0.48	0.57	23.9	0.866	0.259	0.683	0.015	0.006	0.048	0.010
2M	Mean[c]	0.458	15.3*	10.58	2.49	14.5**	33.4	43.3*	1053**	4.25**	0.66**	3.28**	0.17**	0.00**	0.10*	0.02**
	SD	0.0078	0.40	0.241	0.242	0.14	0.52	0.53	41.0	0.937	0.079	0.849	0.049	0.005	0.044	0.008
1F	Mean	0.449	15.3	10.08	2.50	15.2	34.1	44.6	973	3.93	0.73	2.99	0.09	0.00	0.11	0.02
	SD	0.0059	0.39	0.218	0.277	0.20	0.59	0.54	49.8	1.246	0.219	1.097	0.034	0.005	0.031	0.011
2F	Mean[c]	0.463*	15.6	10.28	2.30	15.2	33.7	45.0	1047	4.31	0.75	3.33	0.12	0.01*	0.08	0.03
	SD	0.0172	0.38	0.322	0.389	0.43	1.09	0.35	90.8	1.476	0.317	1.242	0.031	0.004	0.016	0.019

Hct – haematocrit; Hb – haemoglobin; RBC – erythrocyte; Retic – reticulocyte; MCHC – mean cell haemoglobin concentration; MCV – mean cell volume; WBC – total white cell count; N – neutrophils; L – lymphocytes; E – eosinophils; B – basophils; M – monocytes; LUC – large unstained cells; Plt – platelet.

[a] Animals received 3 doses i.m. of MVA.HIVcosv.

[b] 1M – males control; 2M – males receiving MMM; 1F – females control; 2F – females receiving MMM.

[c] *t*-Test: $^{*}p < 0.05$, $^{**}p < 0.01$.

Table 3c
Blood Chemistries–Parameters with statistically significant differences.

Group[a]/Sex		ALT (U/l)	AST (U/l)	Bili (μmol/l)	Urea (mmol/l)	Creat (μmol/l)	Gluc (mmol/l)	Trig (mmol/l)	Na (mmol/l)	K (mmol/l)	Ca (mmol/l)	Phos (mmol/l)	Total Prot (g/l)	Alb (g/l)	Gamma (g/l)	A/G ratio
MMM[b] (*n* = 10 per group)																
1M	Mean	40	54		6.16			1.00					52		1	1.62
	SD	9.3	10.0		0.287			0.241					1.4		0.0	0.119
2M	Mean	56**	91**		7.01*			1.56**					54**		2**	1.50*
	SD	12.9	30.8		1.002			0.519					1.3		0.4	0.079
1F	Mean	40	74						151	4.1	2.48		51	33	1	1.97
	SD	19.8	32.5						1.1	0.36	0.052		1.6	1.6	0.0	0.157
2F	Mean	57*	117**						152*	3.7*	2.56*		55**	35*	3**	1.73**
	SD	16.4	33.5						1.4	0.38	0.463		1.6	1.1	0.5	0.062
DDDC[c] (*n* = 10 per group)																
1M	Mean					10		1.52		4.0			49		30	1.48
	SD					1.3		0.233		0.31			1.2		0.8	0.076
2M	Mean					8*		2.09*		4.3*			48*		28**	1.42*
	SD					1.5		0.768		0.34			1.6		1.0	0.065
1F	Mean			1	8.70		10.12	1.49			2.38	2.31				
	SD			0.0	1.276		1.256	0.624			0.059	0.322				
2F	Mean			2**	6.72**		12.43*	0.91*			2.46**	3.03**				
	SD			0.5	1.028		2.257	0.222			0.066	0.571				

ALT – alanine aminotransferase; AST – aspartate aminotransferase; Bili – Total bilirubin; Creat – creatinine; Gluc – glucose; Trig – triglycerides; Phos – inorganic phosphorus; Alb – Albumin; Gamma – γ-globulin; A/G – albumin/globulin ratio.

[a] 1M – males control; 2M – males receiving MMM; 1F – females control; 2F – females receiving MMM.

[b] Animals received 3 doses i.m. of MVA.HIVcosv.

[c] Animals received 3 doses i.m. of pSG2.HIVcosv DNA followed by 1 dose i.m. of ChAdV63.HIVconsv.

[d] *t*-Test: $^{*}p < 0.05$; $^{**}p < 0.01$.

Table 4
Tissue enlargement.

Group	Treatment	Day of examination	Number of animals/ tissues examined	Organ/tissue examined[a]					
				Spleen		Popliteal lymph node (right)		Inguinal lymph node (right)	
				Male	Female	Male	Female	Male	Female
1	Control	Day 50	10	0	0	0	0	0	0
2	DDDC[b]	Day 50	10	0	5/10	0	0	0	0
3	Control	Day 36	10	0	0	0	0	0	0
4	MMM[c]	Day 36	10	0	0	9/10	8/10	6/10	8/10

[a] The numbers in the table indicate the number of animals in each group with significantly enlarged organs.
[b] Animals received 3 doses i.m. of pSG2.HIVcosv DNA followed by 1 dose i.m. of ChAdV63.HIVconsv.
[c] Animals received 3 doses i.m. of MVA.HIVconsv.

Table 5
Microscopic pathology at the injection site.[a]

Observation	Group 1 (control)		Group 2 (DDDC[b])		Group 3 (control)		Group 4 (MMM[c])	
	Male	Female	Male	Female	Male	Female	Male	Female
Myofibre inflammation	0	0	10/10	9/10	–	–	–	–
Myofibre regeneration	0	0	5/10	8/10	–	–	–	–
Myofibre necrosis/degeneration	0	0	3/10	3/10	–	–	–	–
Interstitial inflammation	0	0	4/10	9/10	–	–	–	–
Intermyofibre/interfascicular/perimysial inflammatory cell infiltrate	–	–	–	–	0	0	10/10	10/10
Dermal inflammatory cell infiltrate	–	–	–	–	4/10	5/10	6/10	8/10
Number of tissues examined	10	10	10	10	10	10	10	10

Table 6
Microscopic changes in the lymph nodes.[a]

Observation	Group 1 (control)		Group 2 (DDDC[b])		Group 3 (control)		Group 4 (MMM[c])									
	Popliteal		Inguinal		Popliteal		Inguinal		Popliteal		Inguinal		Popliteal		Inguinal	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Increased cellularity – generalised	0	1/10	0	0	7/10	6/9	0	0	–	–	–	–	–	–	–	–
Plasmacytosis	0	0	0	0	4/10	1/9	0	1/10	0	0	–	–	4/10	4/10	–	–
Perinodal Inflammation	0	0	0	0	5/10	1/9	0	0	–	–	–	–	–	–	–	–
Increased germinal centre development	–	–	–	–	–	–	–	–	0	0	–	–	9/10	10/10	5/6	7/8
Number of tissues examined	8	10	10	10	10	9	10	10	9	9	0	0	10	10	6	8

[a] The numbers in the table indicate the number of animals in each group with significant microscopic changes.
[b] Animals received 3 doses i.m. of pSG2-HIVcosv DNA followed by 1 dose i.m. of ChAdV63.HIVconsv.
[c] Animals received 3 doses i.m. of MVA.HIVconsv.

Vaccine-elicited Human T Cells Recognizing Conserved Protein Regions Inhibit HIV-1

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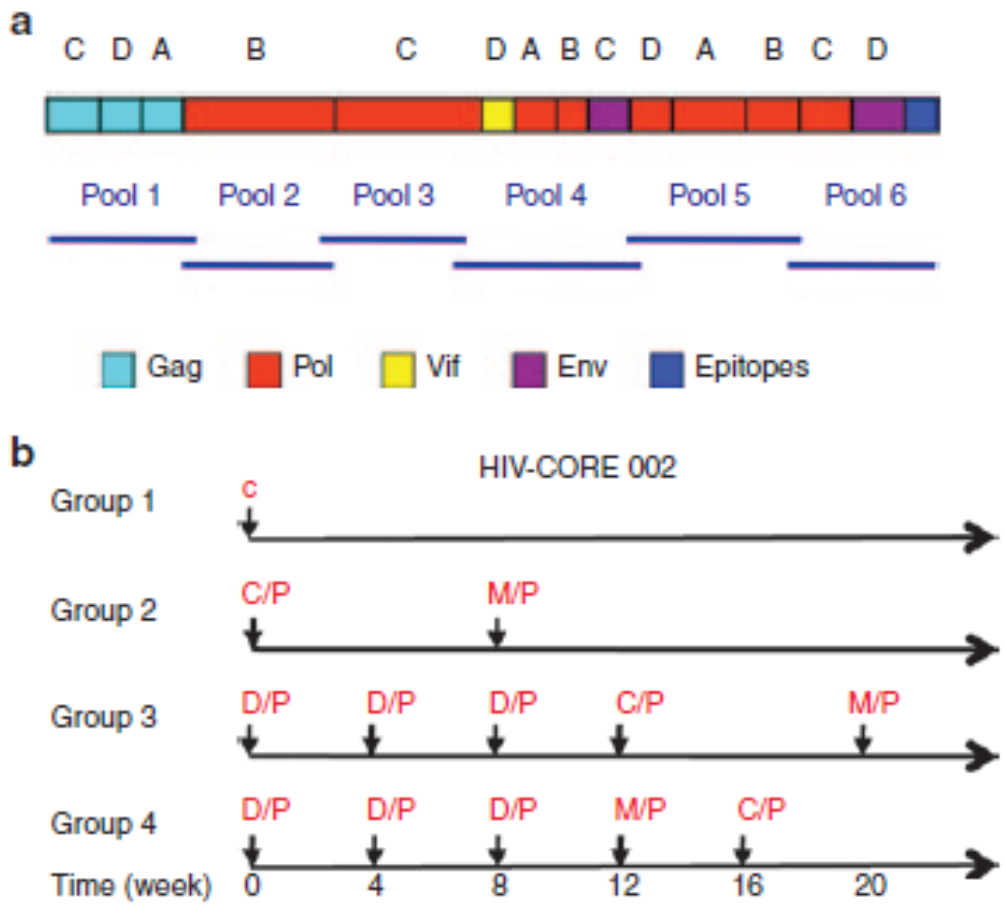
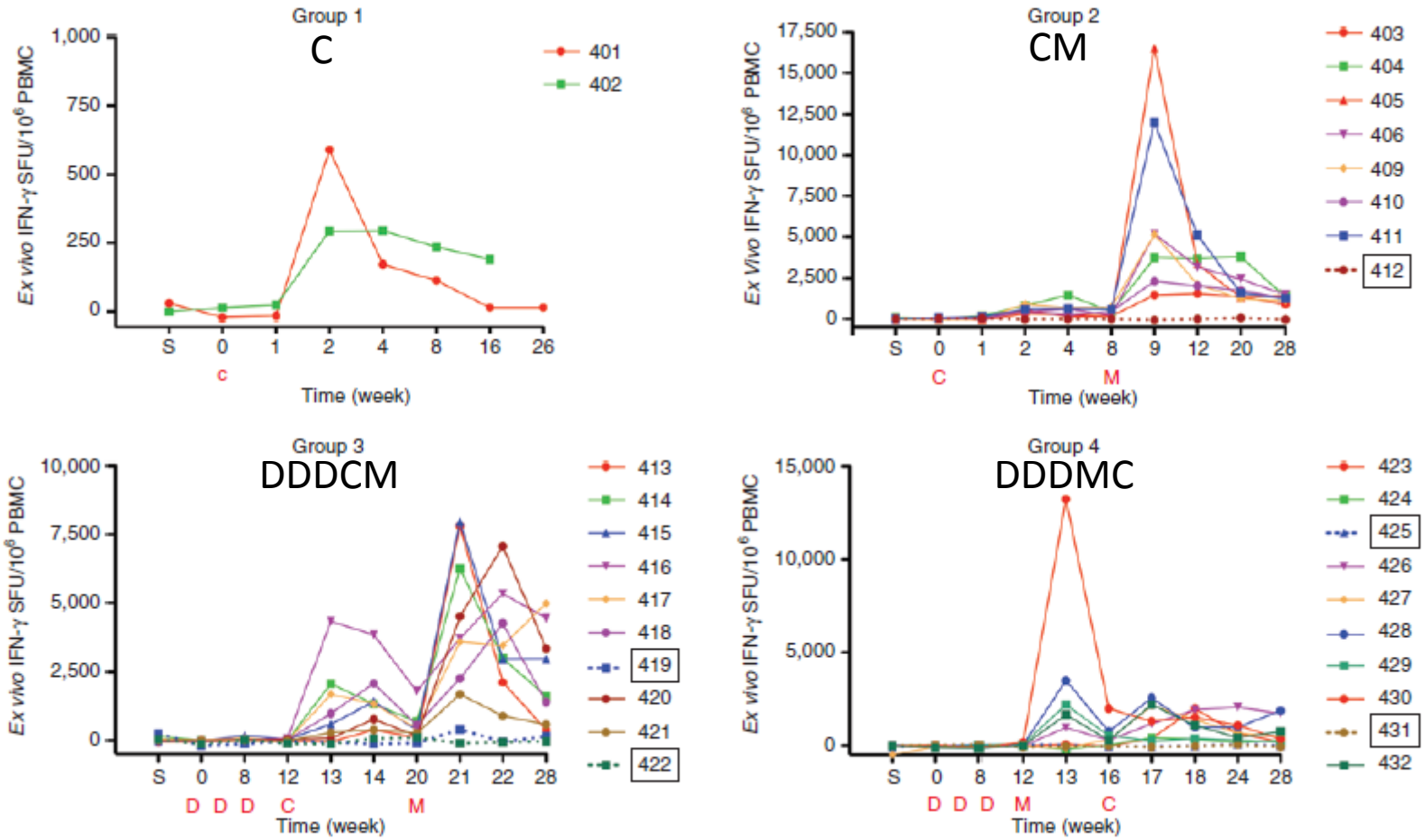
FIRST HUMAN TRIAL (PHASE I)

HIVconsv vaccines induced high frequencies of HIV-1-specific T cells

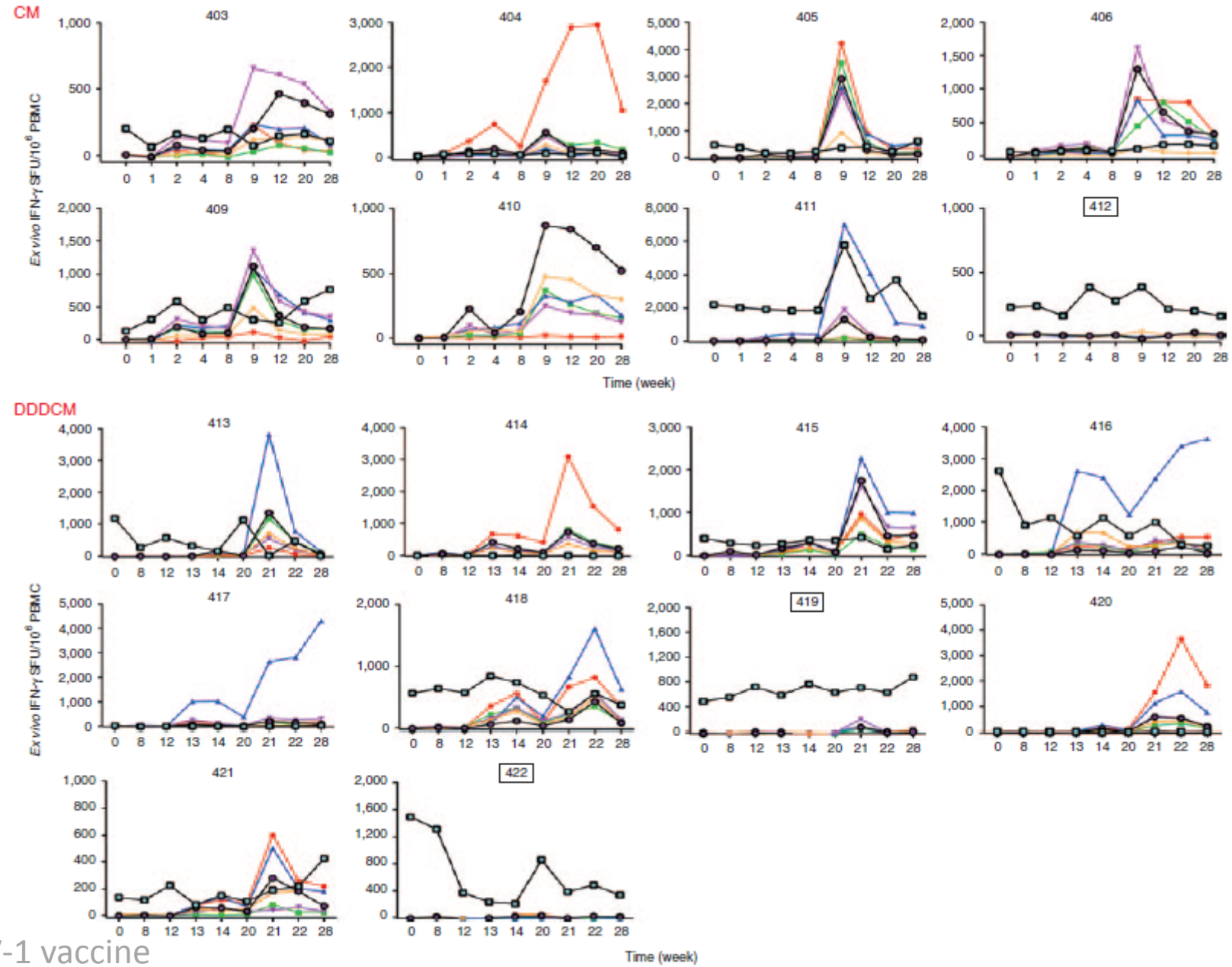


D-pSG2.HIVconsv ,
C-ChAdV63.HIVconsv,
M-MVA.HIVconsv
P-placebo

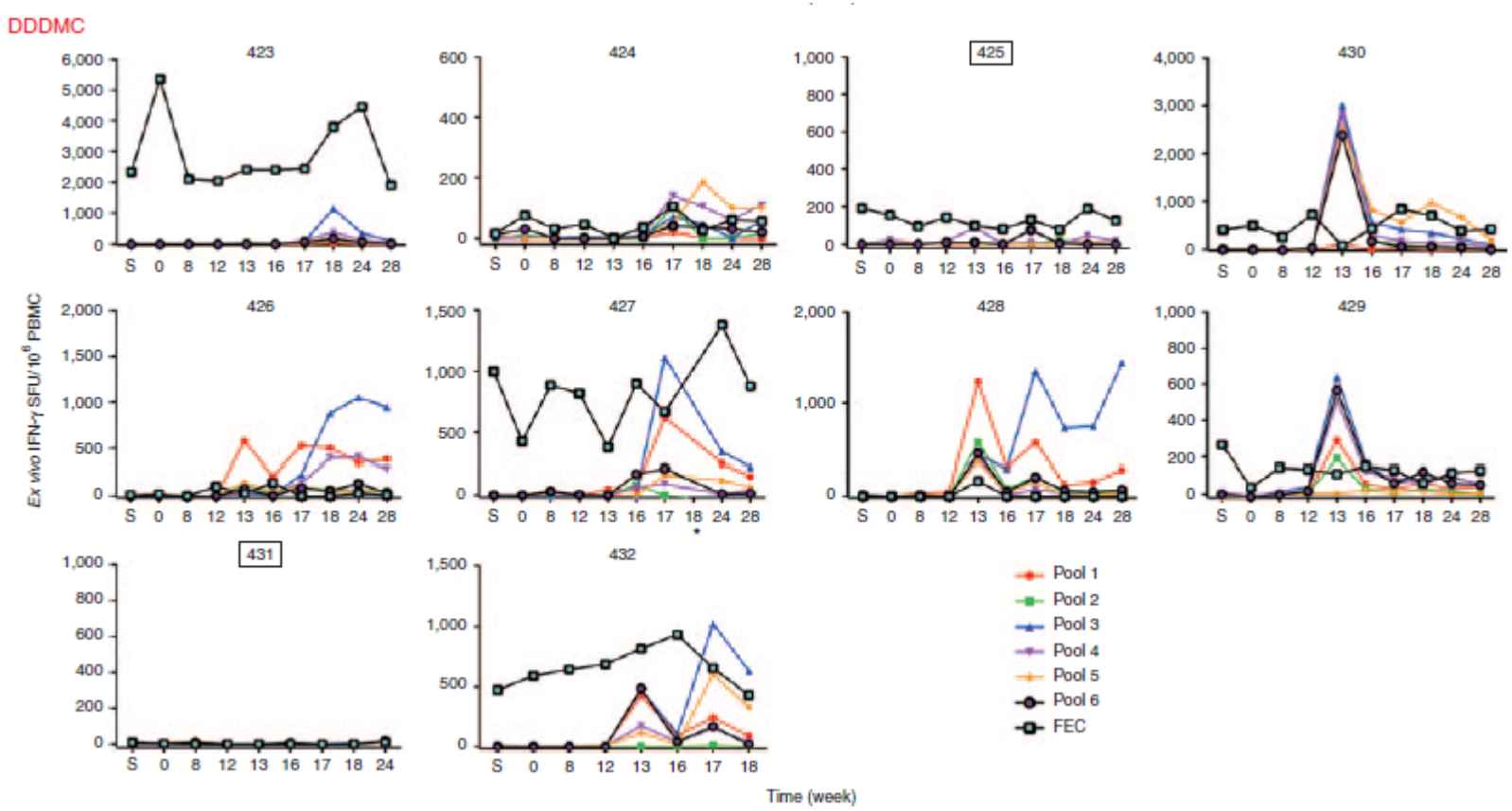
HIVconsv vaccines induced high frequencies of HIV-1-specific T cells



HIVconsv vaccine-elicited T cells were of broad specificities



HIVconsv vaccine-elicited T cells were of broad specificities



HIVconsv vaccine-elicited T cells were of broad specificities

Pool 1

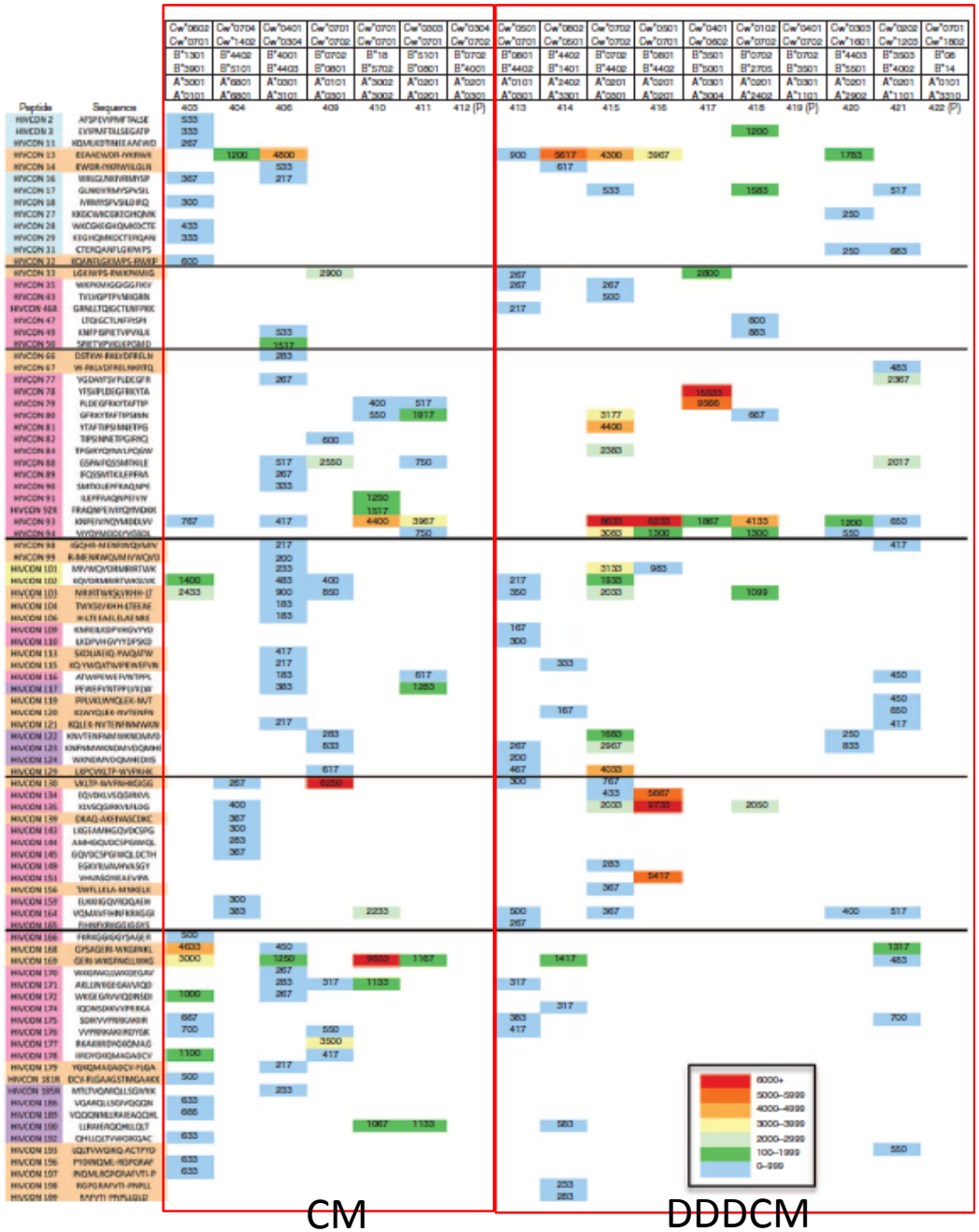
Pool 2

Pool 3

Pool 4

Pool 5

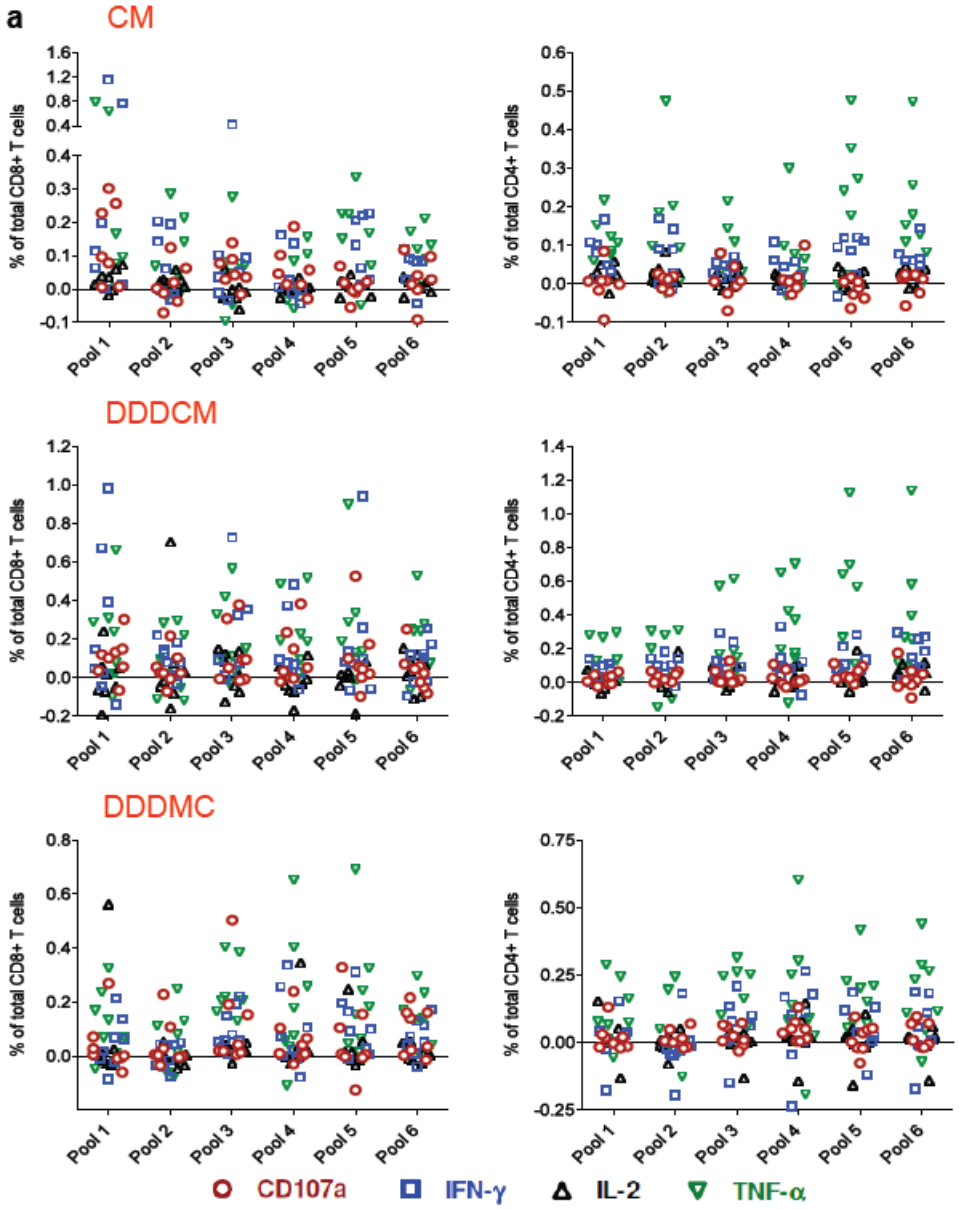
Pool 6



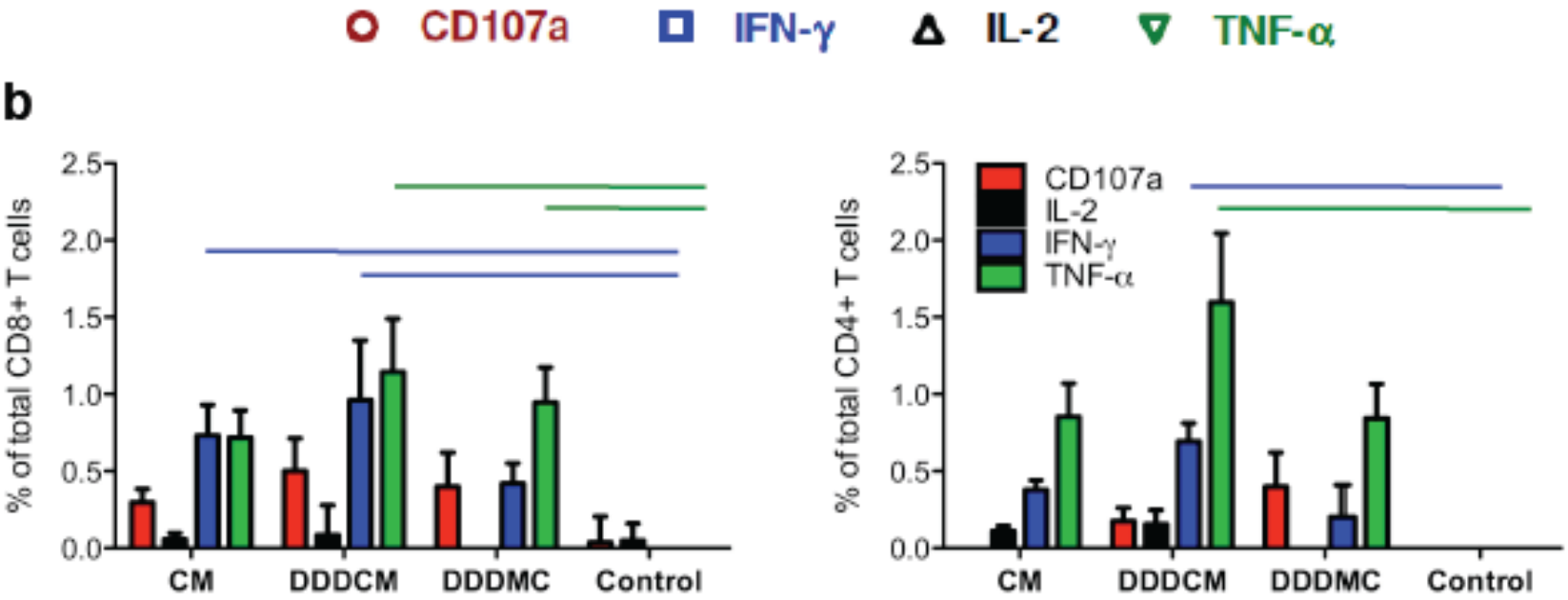
Week 28

blue, Gag
pink, Pol
yellow, Vif
purple, Env
orange background : peptides containing a junction

HIVconsv vaccine-elicited CD4+ and CD8+ T cells were polyfunctional



HIVconsv vaccine-elicited CD4+ and CD8+ T cells were polyfunctional

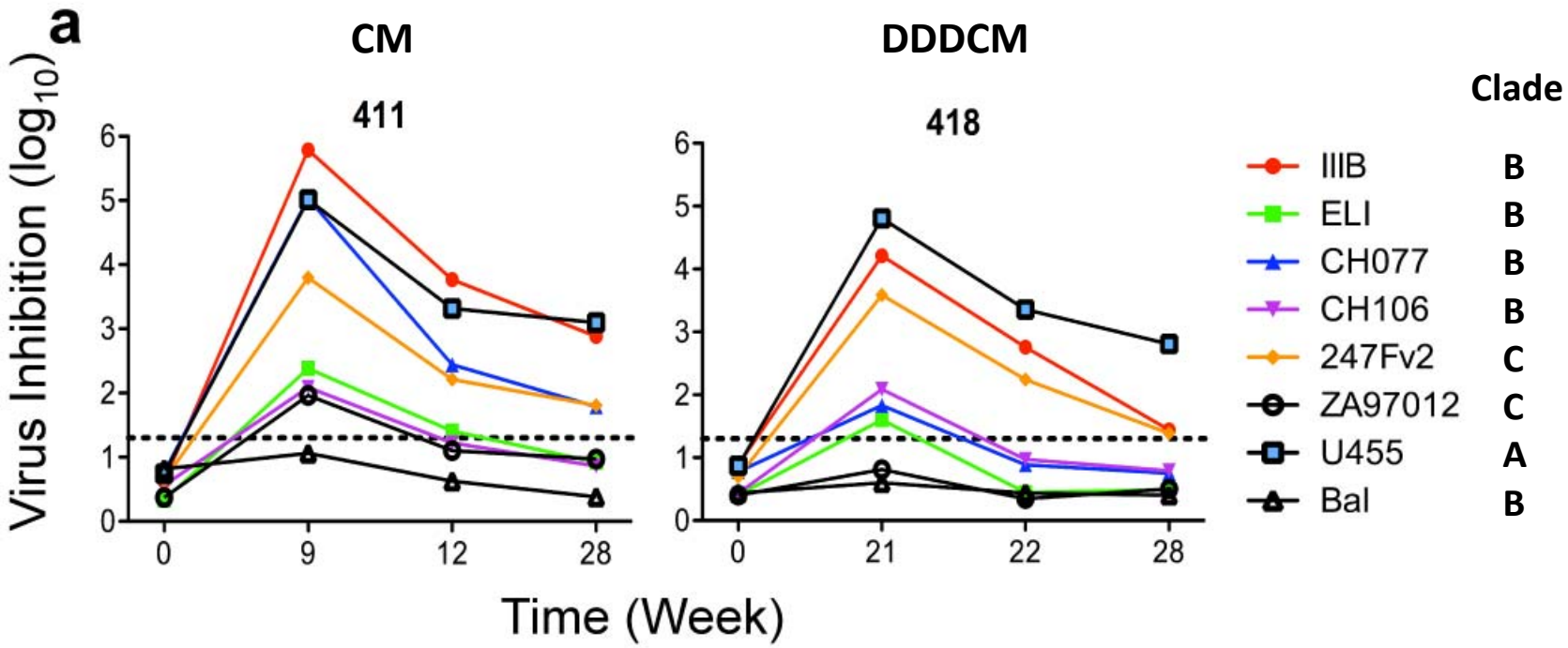


HIVconsv vaccine-elicited T cells inhibited HIV-1 replication *in vitro*

- Exp design

- virus-inhibition assay (VIA)
- vaccine-induced CD8+ T cells **VS** autologous CD4+ T cells
- Different clade of virus tested: ELI, CH077, CH106, 247Fv2, ZA97012, U455, Bal and Nef-mutated IIIB

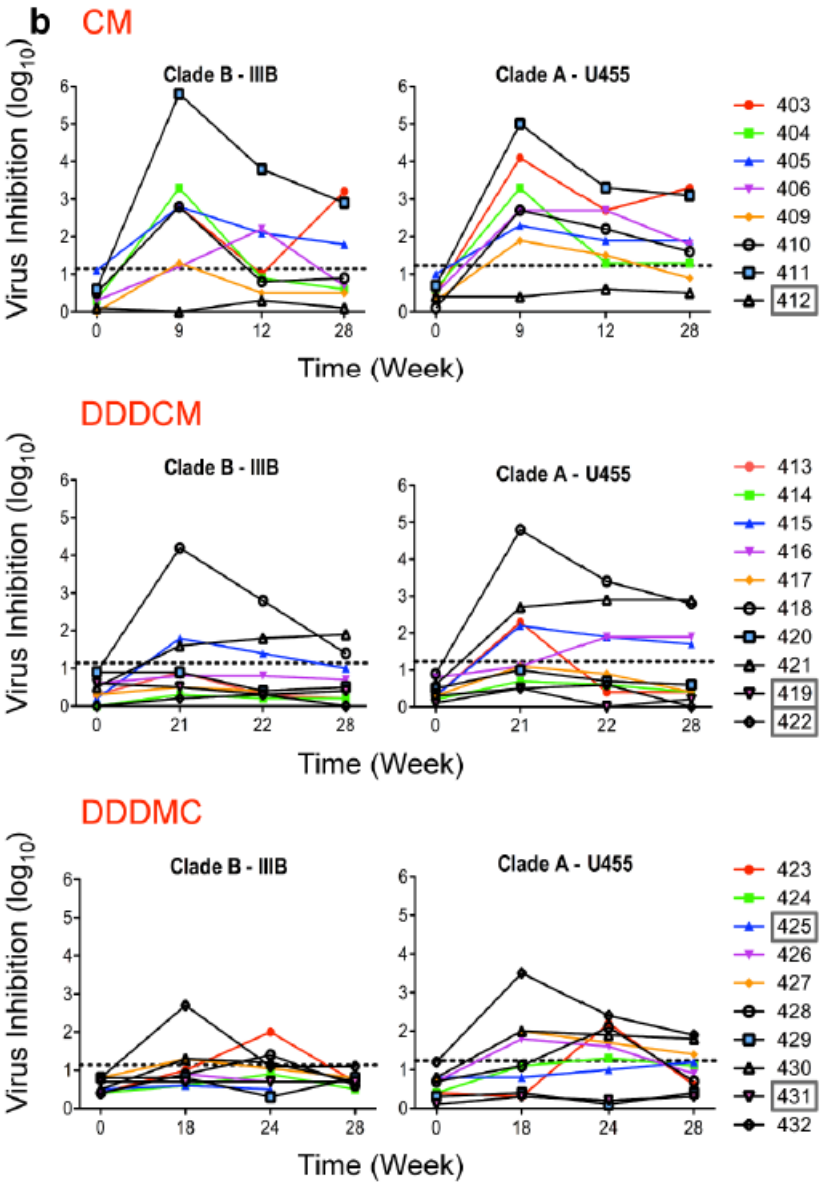
HIVconsv vaccine-elicited T cells inhibited HIV-1 replication *in vitro*



8

6/8

HIVconsv vaccine-elicited T cells inhibited HIV-1 replication *in vitro*

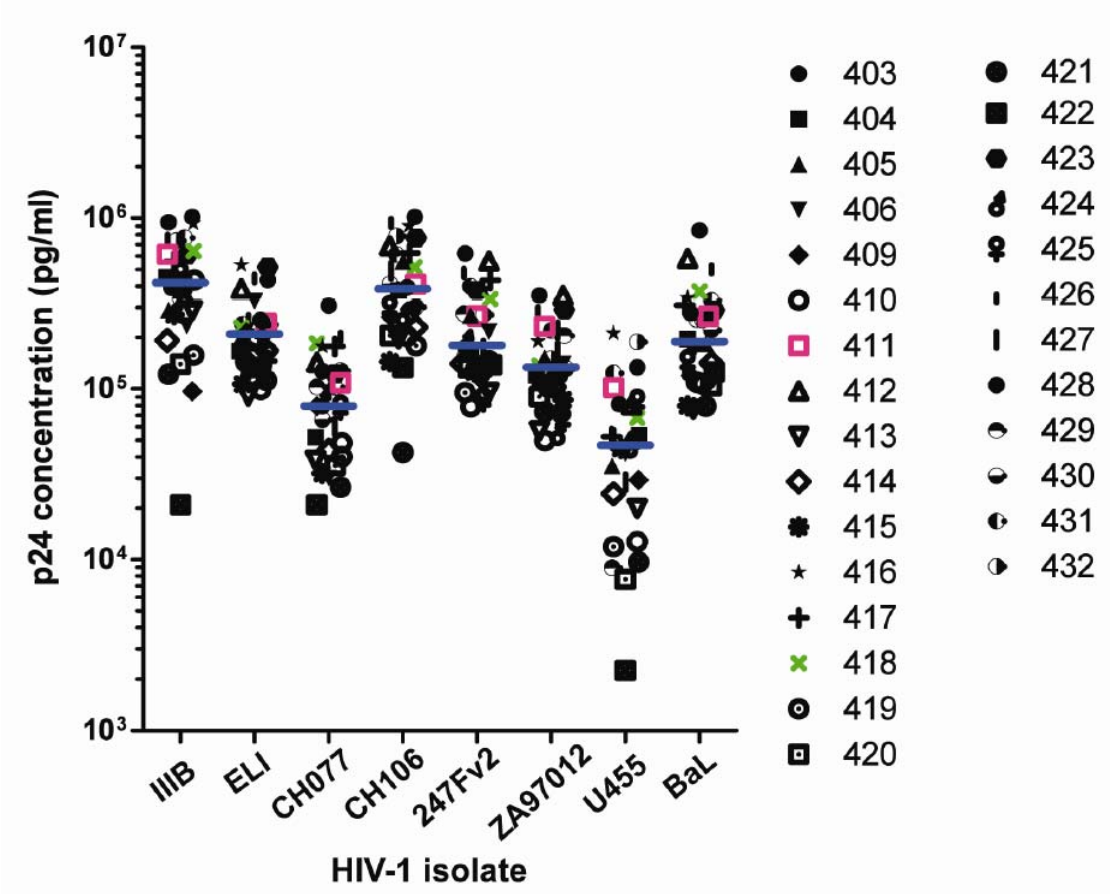


7/7

3/8

5/8

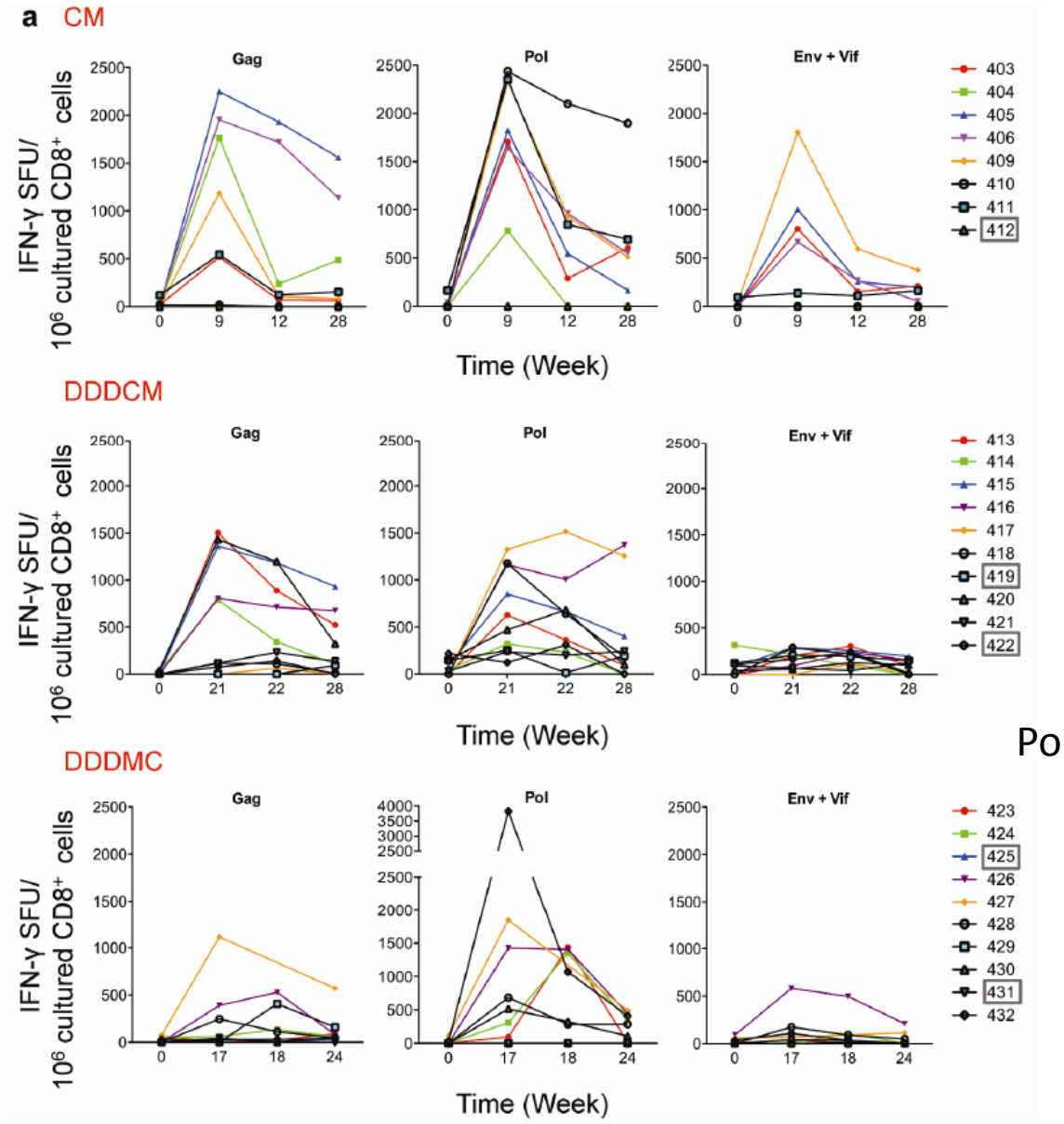
Replicative capacity of HIV-1 isolates in autologous CD4+ cells



Day 13

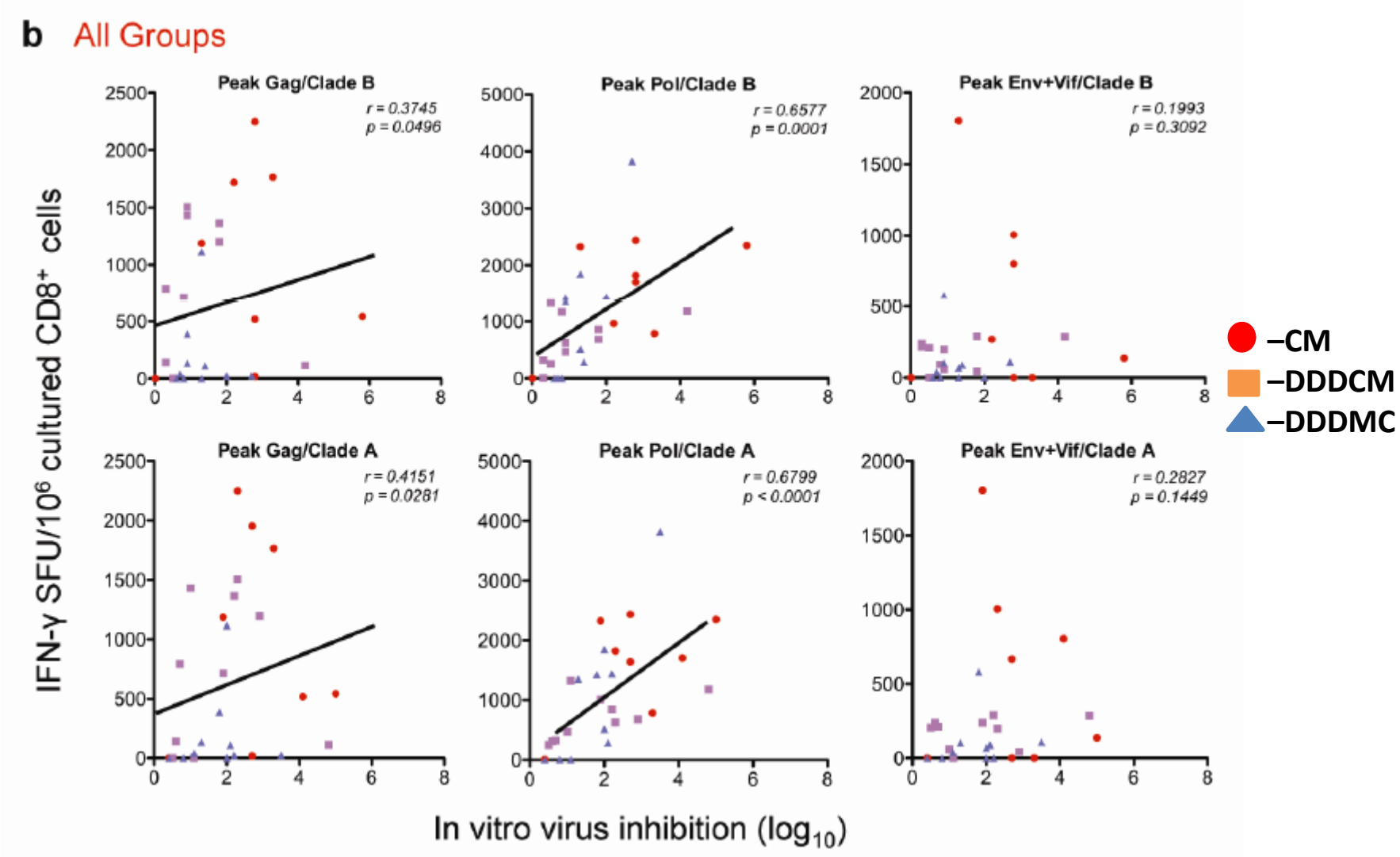
Clade B B B B C C A B

Both Gag- and Pol-specific CD8+ T-cell frequencies correlated with *in vitro* HIV-1 inhibition



Pol (2 pools)

Both Gag- and Pol-specific CD8+ T-cell frequencies correlated with *in vitro* HIV-1 inhibition



Summary

- Chimeric immunogen HIVconsv made of consensus clade sequences, which may not be present in natural isolates, elicited T cells that recognized HIV-1-infected cells.

- The CM and DDDCM regimens induced transgene-specific, IFN-γ–producing T cells, which (i) reached **unprecedented high median** total frequencies of 5.2 k and 5.8 k SFU/106 PBMC, respectively; (ii) included both **CD8+ and CD4+ T**-cell subpopulations;(iii) were **broadly specific**; (iv) produced **multiple intercellular signaling molecules**; (v) **proliferated** to recall antigens; and (vi) showed efficacy *in vitro* by **inhibiting HIV-1 replication** in cultured autologous cells.

- Correlation of *ex vivo* IFN-g ELISPOT assay frequencies with virus inhibition.

- Pol specific CD8+ T-cell responses correlated as well as or better than the Gag-specific responses with virus inhibition.

Outlook

- HIVconsv-specific responses were detected in 100% of vaccinees and recognized an average of 8 naturally subdominant HIV-1 epitopes, which originated from conserved regions common to many circulating viruses.
- Some modification of the delivery regimen may be needed.
- Demonstrate protection of humans against HIV-1 infection in conjunction with a vaccine that elicits effective anti-Env antibodies.


Thank You!

Overview: Past HIV-1 vaccine trials

envelope glycoprotein gp120 vaccine	AIDSVAX B/E gp120 clinical trial: high-risk cohort	reported 2006 [1]: no protective effect
vaccine targeting Gag, Pol & Nef, vectored by adenovirus type 5, aiming to induce CD8+ T cell response	STEP clinical trial (MRK rAd5 vaccine)	reported 2008 [2]: no protective effect; may even increase risk (vector safety?)
prime with ALVAC-HIV recombinant canarypox vector vaccine + boost with AIDSVAX B/E gp120	RV144 clinical trial: lower-risk cohort	reported 2009 [3]: estimated 31% protective effect

References:

- *Review*: McMichael & Haynes (2012)
- *Original reports:* [1] Pitisuttithum *et al. J Infect Dis* 194:1661-1671 (2006); [2] Buchbinder *et al. Lancet* 372:1881-1893 (2008); [3] Rerks-Ngarm *et al. N Engl J Med* 361:2209-2220 (2009)