Impact of RNA- and Codon Optimization on Safety and Immunogenicity of HIV Candidate Vaccines

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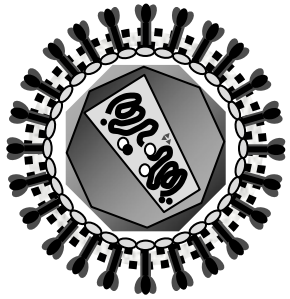
Integrated Vaccine Strategies

HIV Clades

- A (Africa)
- B (Europe, N & S-America)
- **C (Asia, . …)**
- E (Asia)
- F (Africa)
- G (South America, Africa)

+

Targets of immune response



- envelope proteins: **Env**
- structural proteins: **Gag, Pol**
- regulatory proteins: Tat, Rev, **Nef**
- accessory proteins: Vpr

+

Immune response

- neutralizing AB
- mucosal immunity
- CD4+ T cell response
- CD8+ CTLs
- broad, cross clade,
- high quality, polyfunctional
- long term memory

immunogen design
molecular virology

Delivery / Combination of Multivalent HIV/AIDS Vaccine Strategies

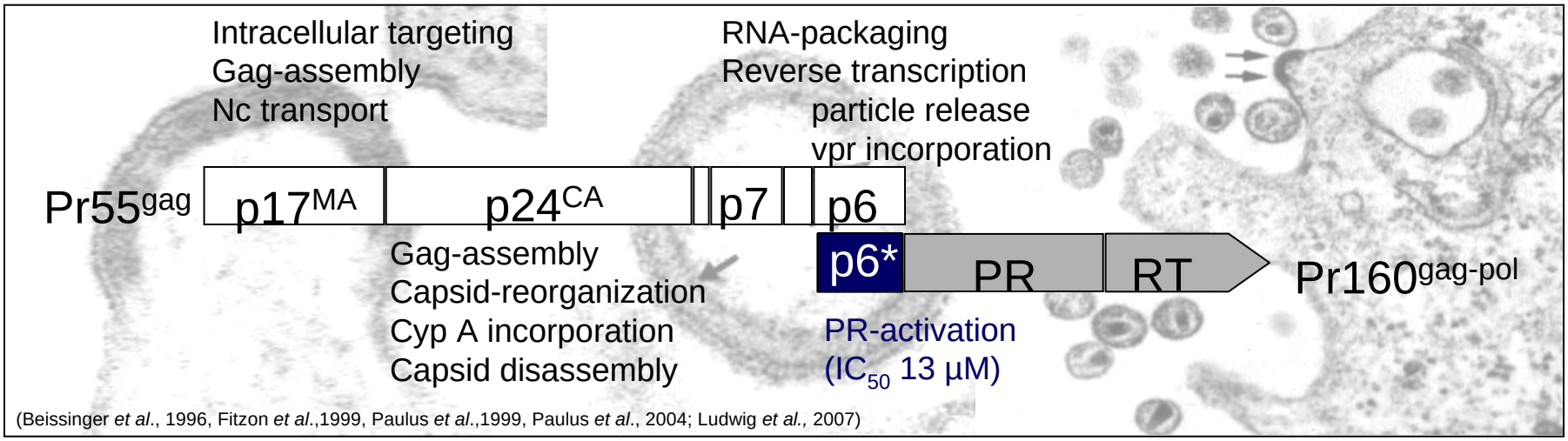
Proteins | **Peptides** | **Novel Adjuvants (CpG, PEI, MF59)** | **DNA** | **Viral Vectors** rAd, **NYVAC** | **Bacterial Vectors** | **Pseudovirions**

?

basic immunology / mice
preclinic / rhesus macaques
clinic / phase 1/2

(Bojak, Deml & Wagner, 2002)

Pr55^{gag} – Necessary Component of HIV Vaccines

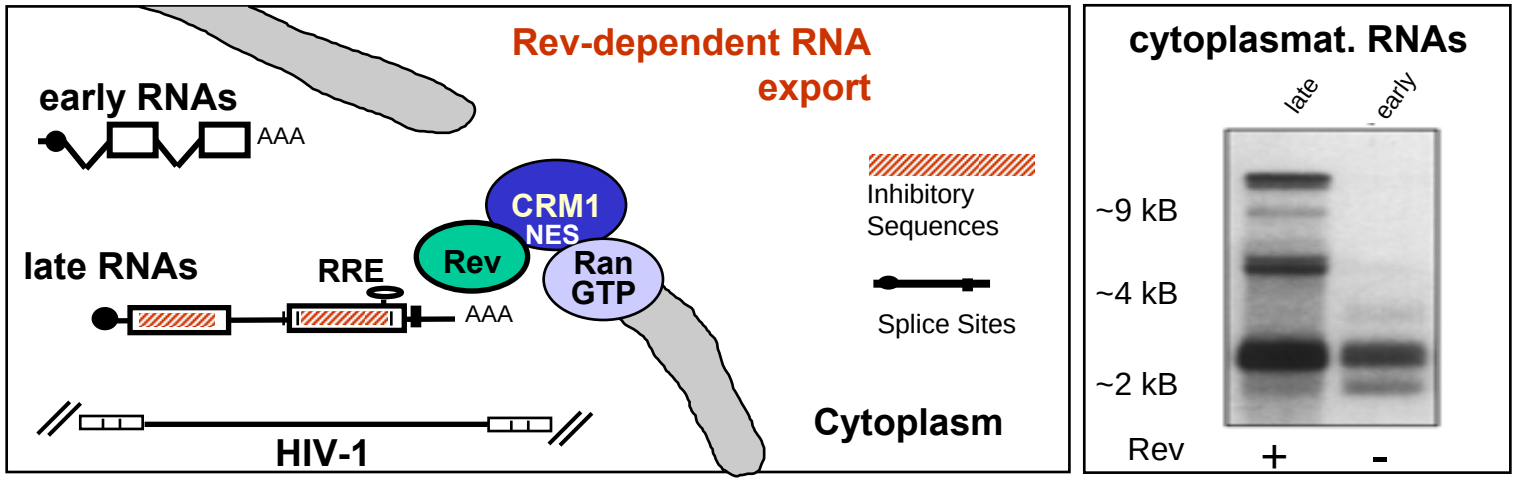


(Beissinger *et al*., 1996, Fitzon *et al*.,1999, Paulus *et al*.,1999, Paulus *et al*., 2004; Ludwig *et al.,* 2007)

- Patients with strong Gag-Helper/CTL-Response control acute HIV infection better (Rosenberg *et al*., 1997; Ogg *et al*., 1999, many others)
- LTNP HIV infected individuals control HIV replication by T cells recognizing highly conserved epitopes within Gag (Harrer *et al*., 1998, Wagner *et al*., 1999, many others)
- Recombinant and chimeric Gag VLPs induce Th1 / CTL responses in Balb/C mice; *Cross priming* (Wagner *et al*., 1993, 1994, 1996; Bojak et al., 2002, 2003, 2004, Deml *et al.*,1997a,b, 2005)
- Gag VLP partially protective in Rhesus monkey model (Wagner et al., 1998; Notka et al., 1999a, b, Ludwig & Wagner, 2007)

➔ **Gag specific T-cells have been linked to better control of HIV replication**

Late Lentiviral Gene Expression is Complex

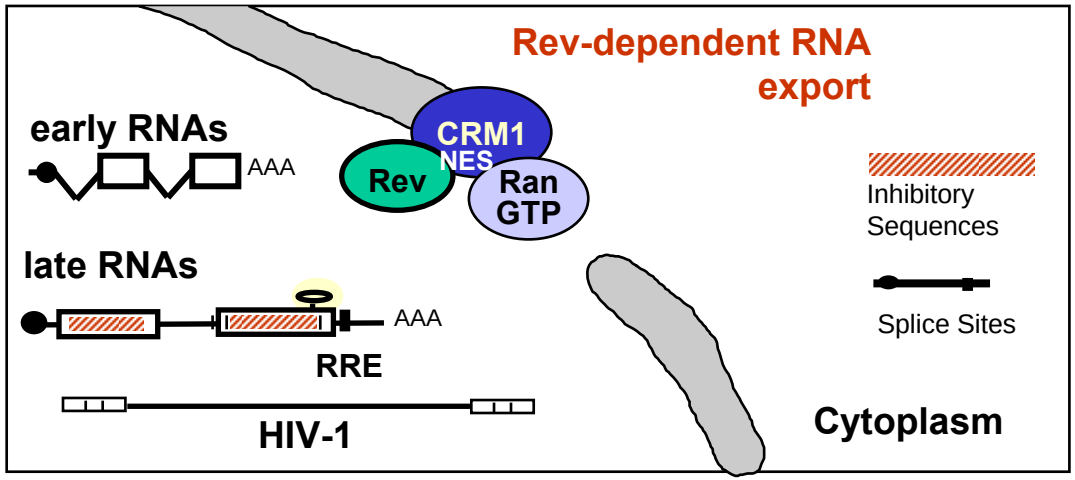


strong splice donor and weak splice acceptors capture RNAs within splicing machinery

cis-located AU-rich repressor elements (INS) retain RNAs within the nucleus

Intrinsic risks: Ψ-sequence, recombination, RRE/Rev, ...

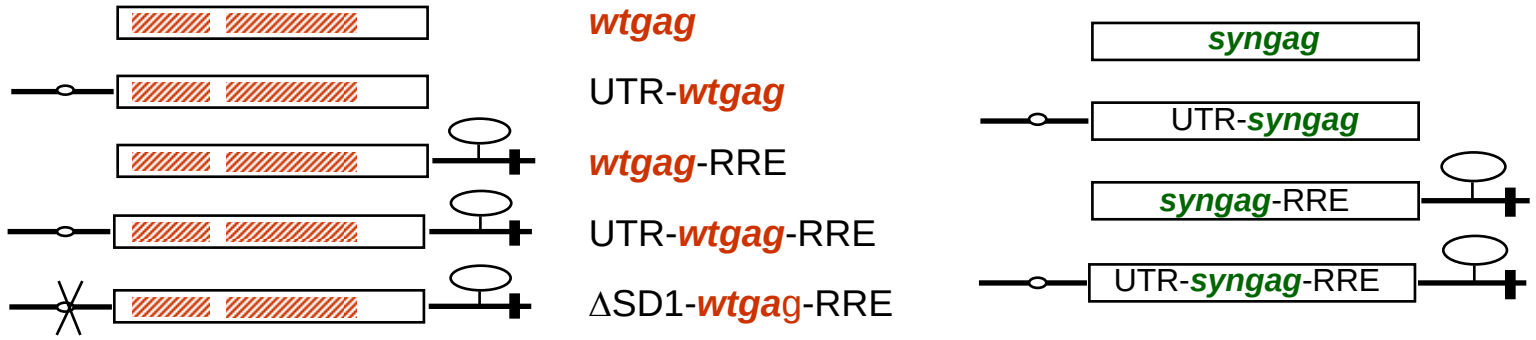
Understanding Late Lentiviral Gene Expression



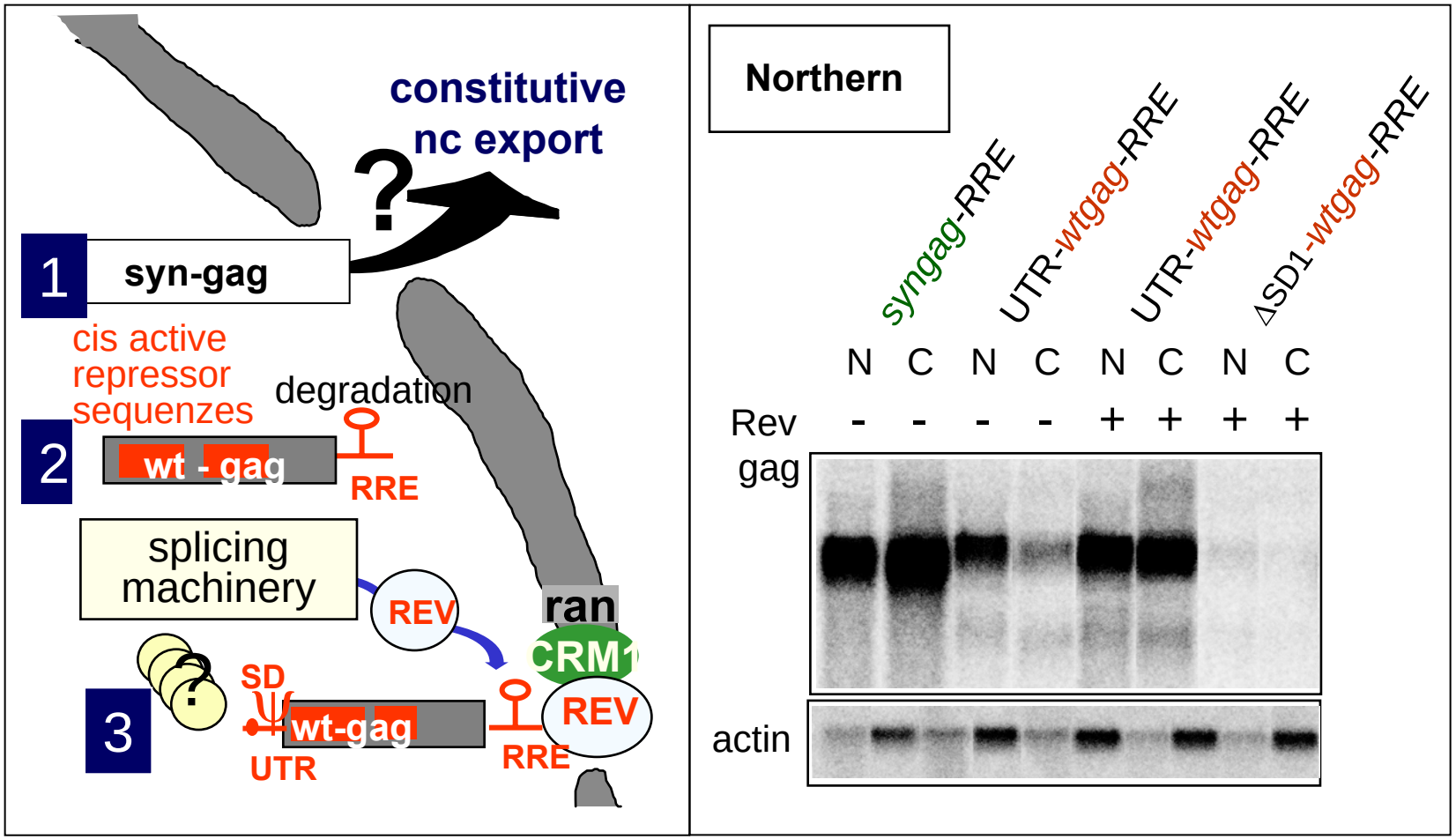
AA	mammals	HIV-1
Ala	GCC	GCA
Arg	AGG	AGA
Asn	AAC	AAT
Cys	TGC	TGT
Gln	CAG	CAA
Glu	GAG	GAA
Gly	GGC	GGA

Influence of various cis-acting sequences [INS, UTR (SD), RRE (SA)]

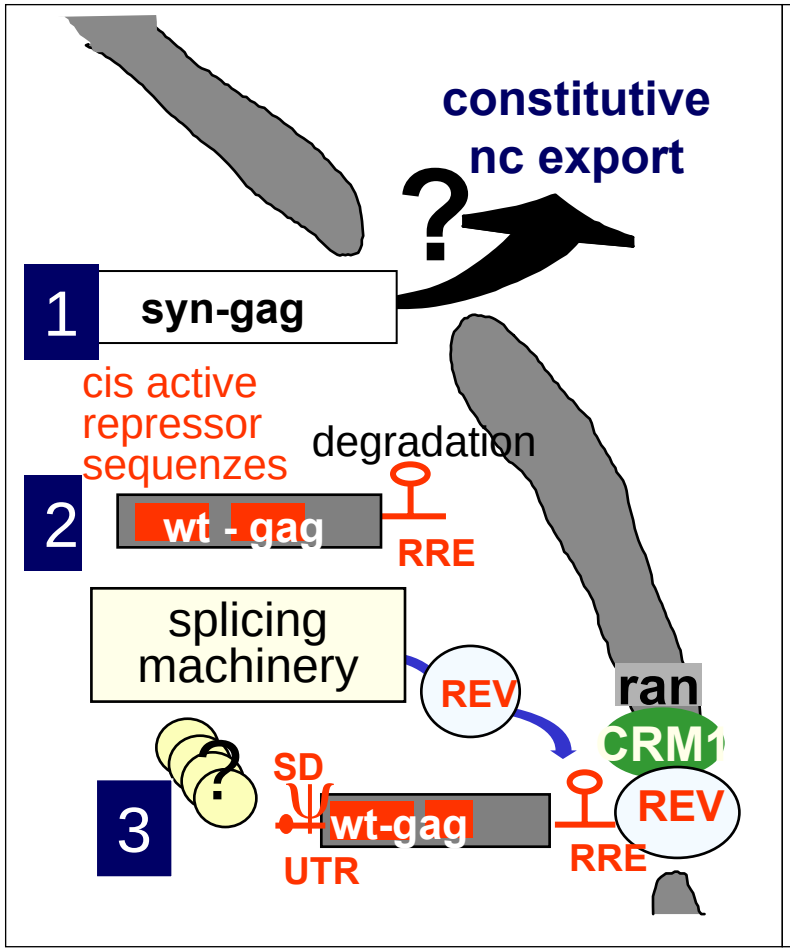
GENEART THE GENE OF YOUR CHOICE



Facilitating Late Lentiviral Gene Expression



Consequencs of Modulated Late Lentiviral Gene Expression



HIV Vaccines

- facilitated gag-pol expression
- RNA nuclear export 50x
- no Rev, no RRE
- no 5'-UTR, no Ψ

Risk for Recombination
RNA Packaging

Increased safety ?
Increased yields
Enhanced Immunogenicity ?

- Protein Production (CHO, PerC6)
- DNA Vaccines
- Bacterial DNA Delivery (Listeria)
- Adenoviral Vectors
- Herpesviral Vectors (EHV)
- Lentiviral Vectors
- **Poxviral Vectors?**

Graf et al., 2000; Wagner et al., 2000; Deml *et al.*, 2001; Bojak *et al.*, 2003, Wild *et al.*, 2004, 2007

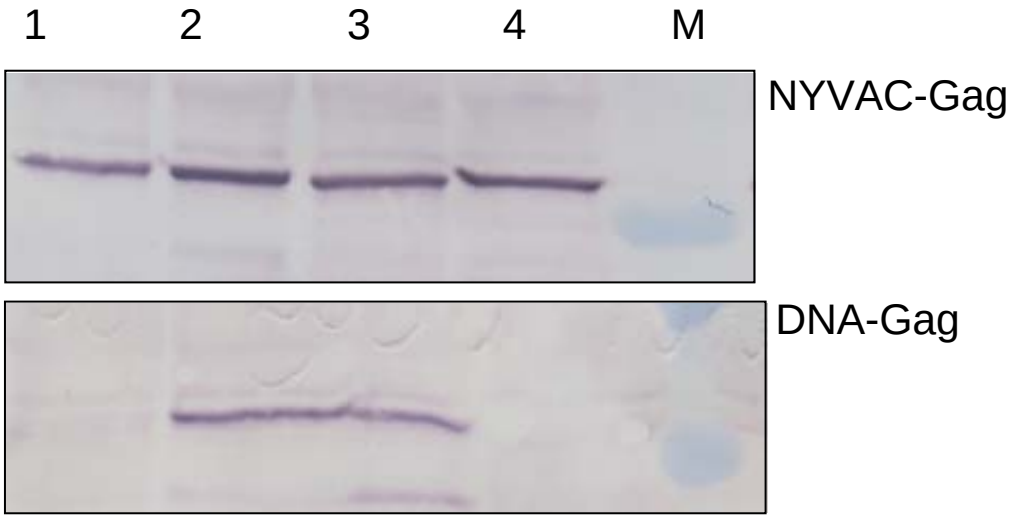
Impact of RNA & Codon Optimization in Poxviral Vectors

1 wt - gag

2 hu opt - gag

3 hu opt cpg_{max}-gag

4 vaccinia - gag



WB - Antibody: CD-4/1 (anti p24 CA)

NYVAC: <u>No</u> major impact of RNA & codon optimization on transgene expression

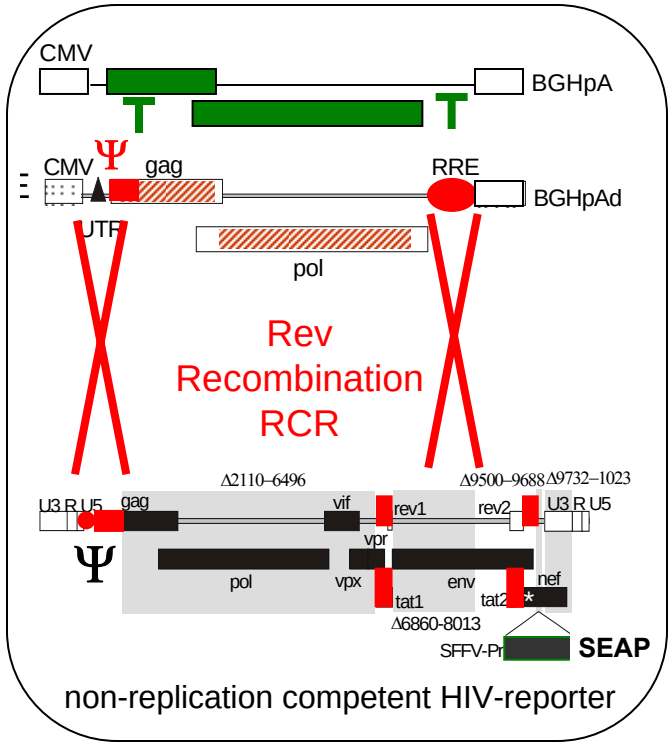
DNA: <u>Significant</u> impact of RNA & codon optimization on transgene expression

Conclusion: RNA- and codon-optimization recommended if various vector systems shall be compared / combined

Reduced Risk for Generating Replication Competent Recombinants

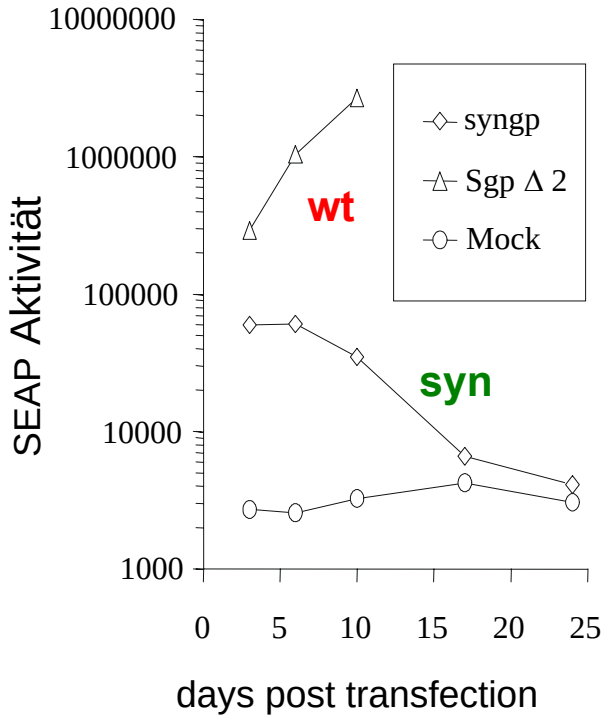
...following RNA- and Codon-Optimization

- 2-Plasmid-system
- Transfervector gagpol^{-}/Env^{+}
- packaging construct wt vs syn
- cotransfection and harvest particles



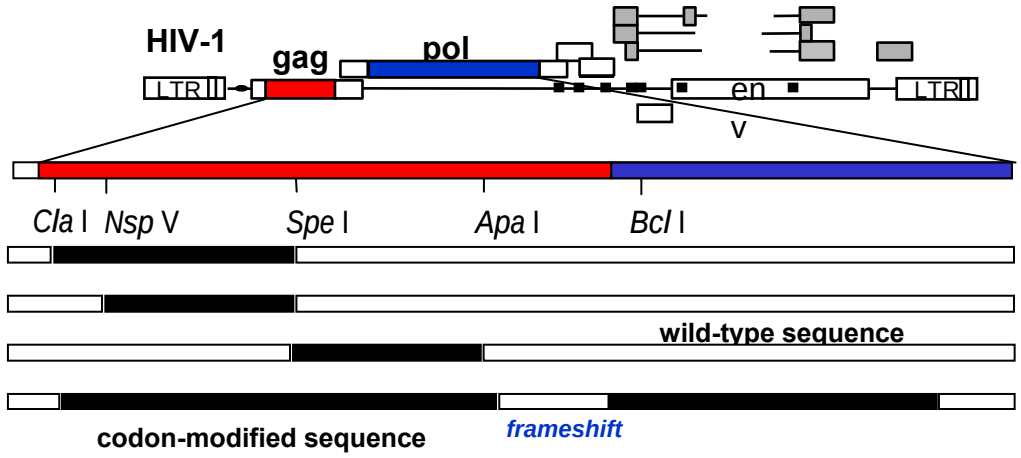
non-replication competent HIV-reporter

- Permissive CD4+ cell culture system
- Replication post recombination
- Tat driven SEAP expression



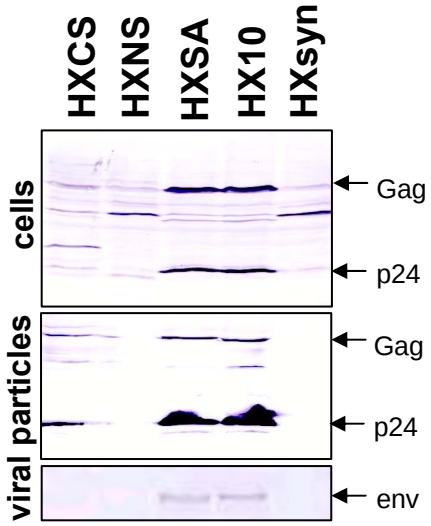
Reduced Replicative Capacity of Engineered Recombinant HIVs

chimeric viruses

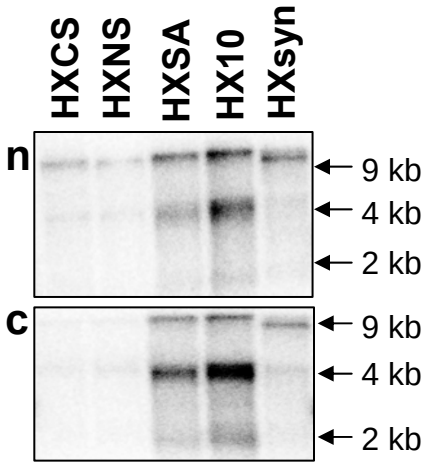


Mutant:	Codon-optimized region (gag ATG=112):	
HXCS	5‘ gag	(bp 154-830)
HXNS	5‘ gag	(bp 337-830)
HXSA	3‘ gag	(bp 830-1333)
HX10	-----	
Hxsyn	*gag/pol*	(bp 154-1333...)

Processing & maturation



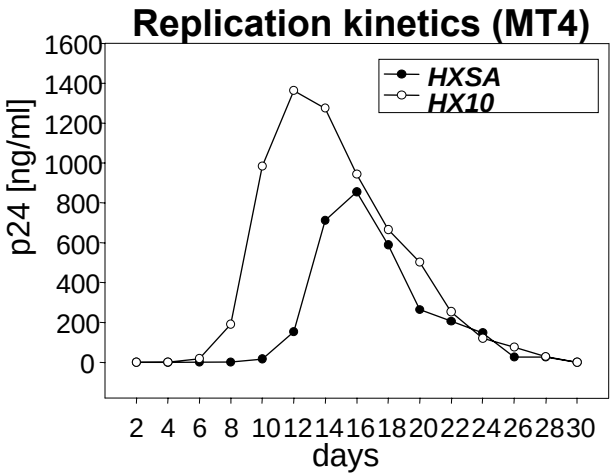
RNA splicing & export



12 h p.t.

(Wagner *et al.*, 2000; Steck *et al.*, in prep.)

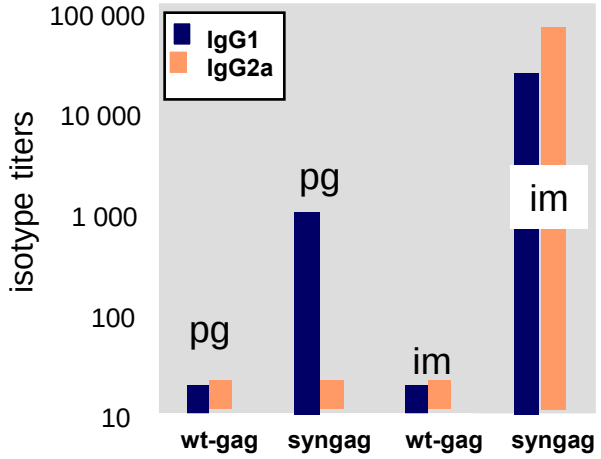
Viral infectivity

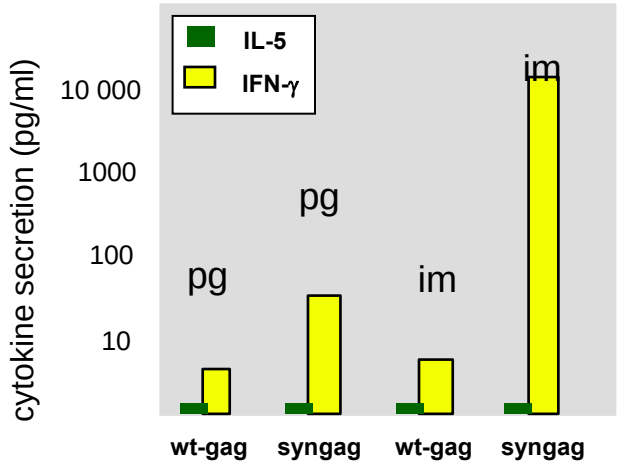


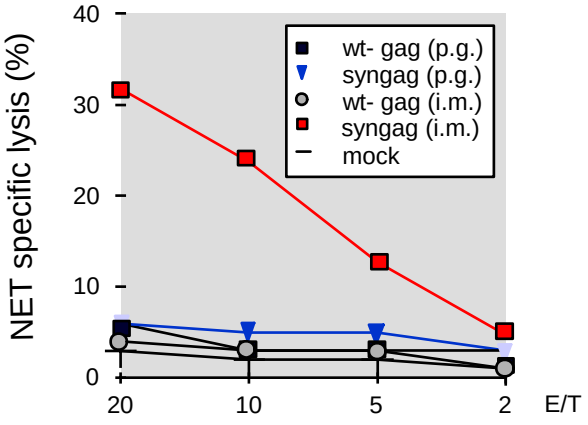
Safety Profiles

RNA and Codon Optimization DNA Vaccines

Increased immunogenicity in BALB/c mice





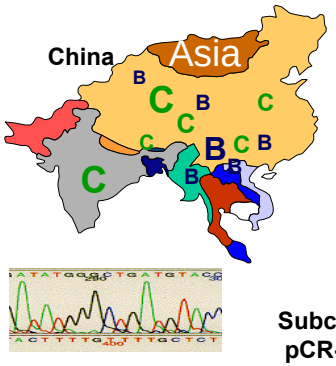


Wt-Gag:
No Gag specific antibodies, IFN-g or CTLs detected

Syn Gag:
High levels of gag specific antibodies and IFN-g; CTL induction

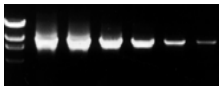
Im (Th1, CTL) **>>> pg** (Th2)

Molecular Epidemiology: Prerequisite for Immunogen Design

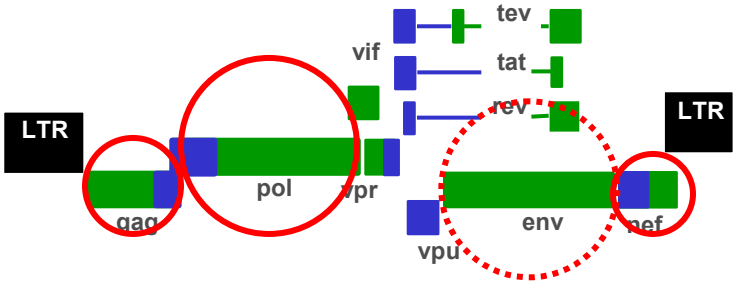


Prevalence
Incidence
Virus population
Regional clustering
Host genetic background

Subcloning
pCR-script



Sequence analysis of C (CN54)

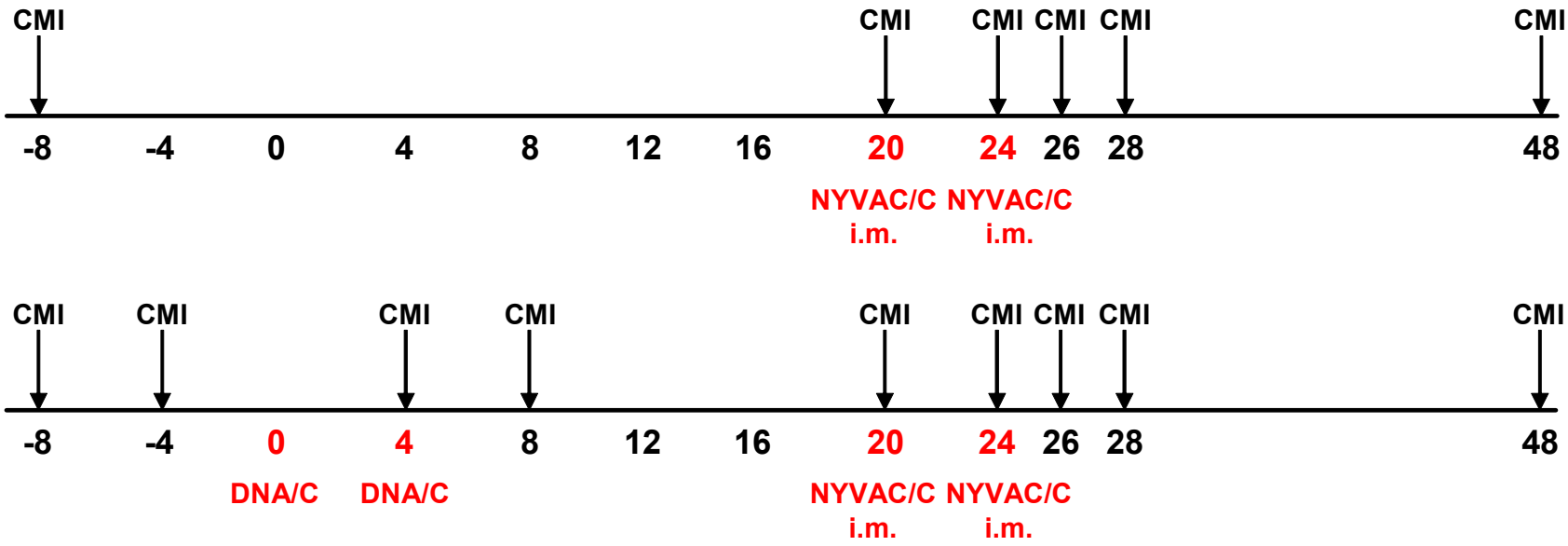


Graf et al., 1998, Su et al., 2000

GENEART THE GENE OF YOUR CHOICE	Clade	Delivery	Status	Developer / Manufacturer
GagPolNef	C (CN54)	DNA-C	GMP, B, T, I, Fill.	Wagner / Wolf / Cobra
Env	C (CN54)	DNA-C	GMP, B, T, I, Fill.	Wagner / Wolf / Cobra
GagPolNef / Env	C (CN54)	NYVAC-C	GMP, B, T, I, Fill.	Sanofi Pasteur
GagPolNef / Env	C (CN54)	MVA-C	GMP in progress	Esteban
GagPolNef / Env	C (CN54)	vTT-C	in progress	Y. Shao / S-CDC
GagPolNef / Env	B	NYVAC-B	GMP, B, T, I, Fill.	Sanofi Pasteur
GagPolNef / Env	B	MVA-B	GMP, B, T, I, Fill.	Esteban

In vivo Studies in Rhesus Monkeys

Mimicking a Human Phase I Trial

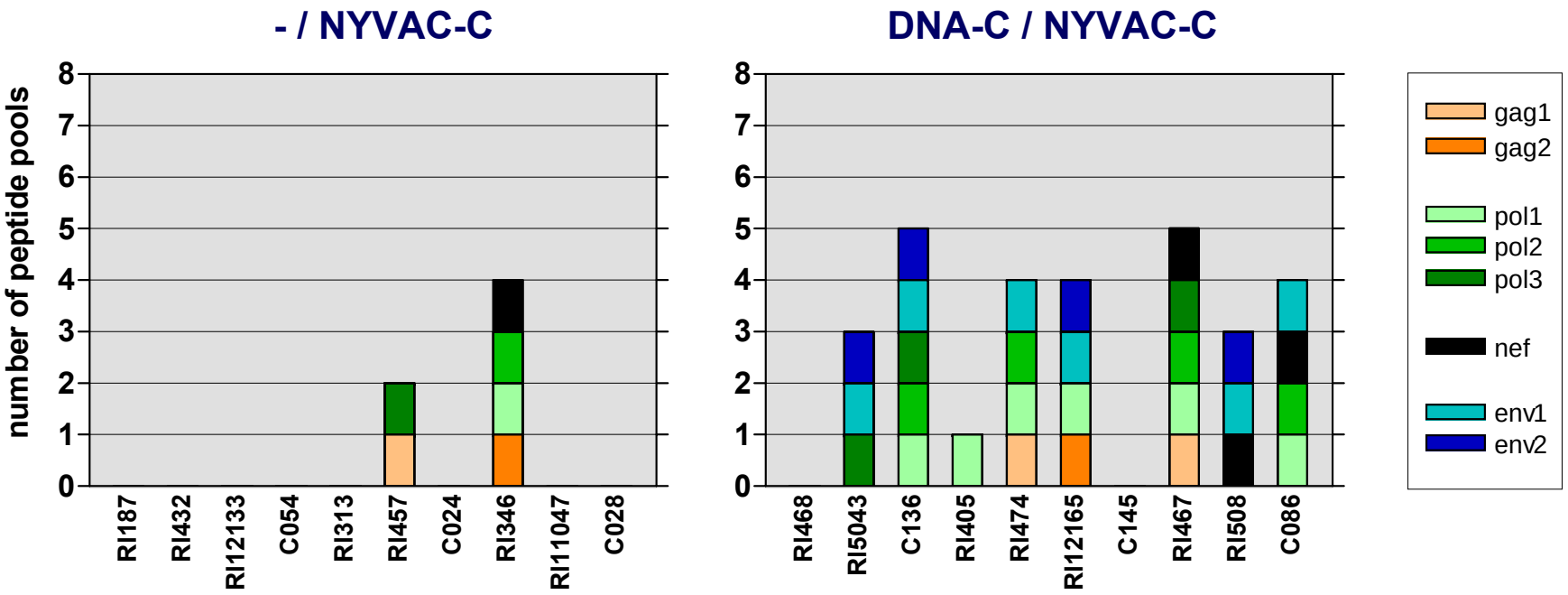


10 monkeys per group

In vivo Studies in Rhesus Monkeys

Mimicking a Human Phase I Trial

Antigen-specific IFNγ release at week 24 (4 weeks after 1st NYVAC)

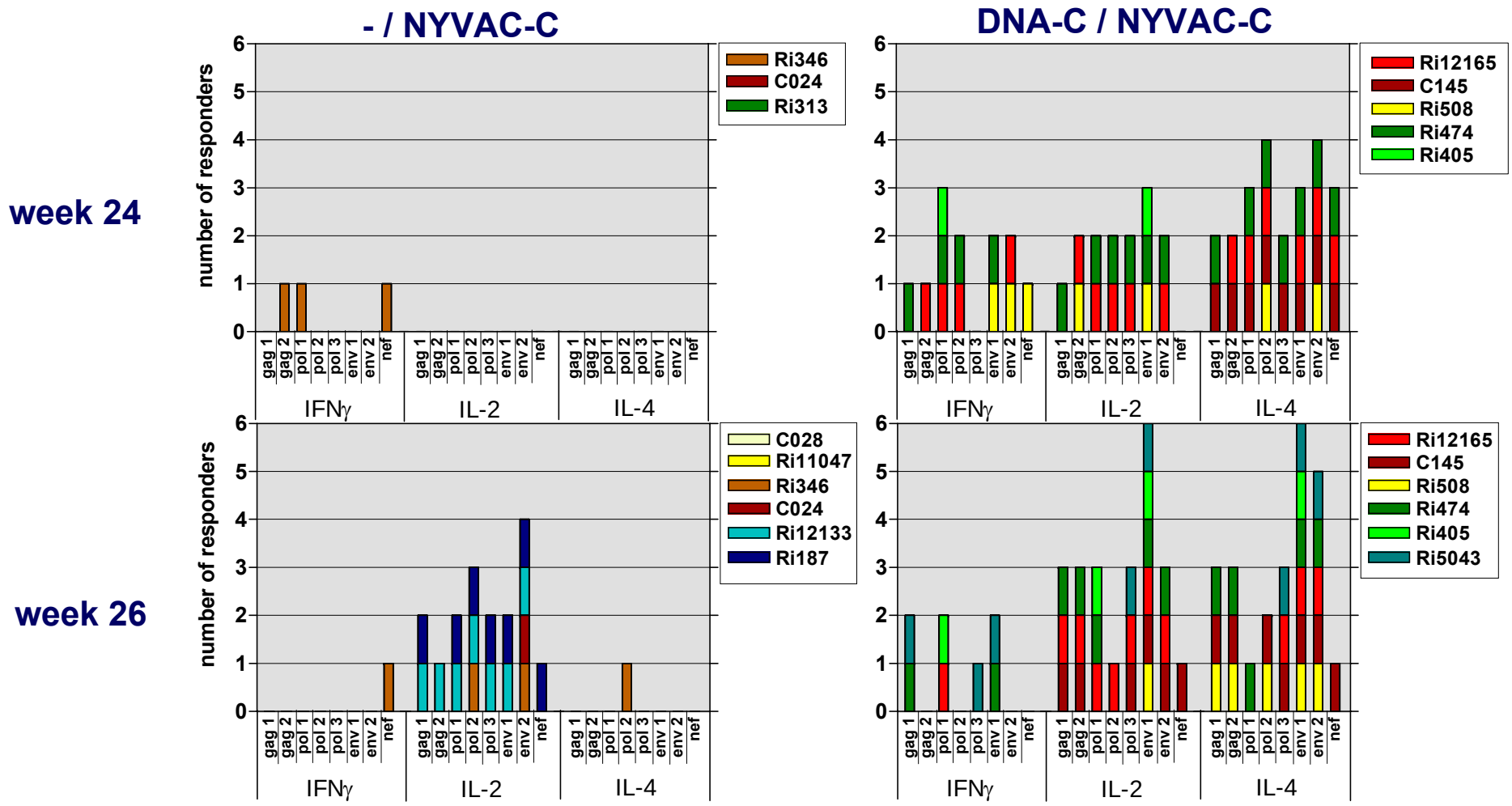


2/10 respond

8/10 respond
several pools recognised

P. Mooij, J. Heeney *et al.*

In vivo Studies in Rhesus Monkeys



P. Mooij, J. Heeney *et al.*

EV02: Study Design – HIV Negative Volunteers

Objective: **Determine the impact of DNA-C versus NYVAC-C alone on priming T-cell responses**

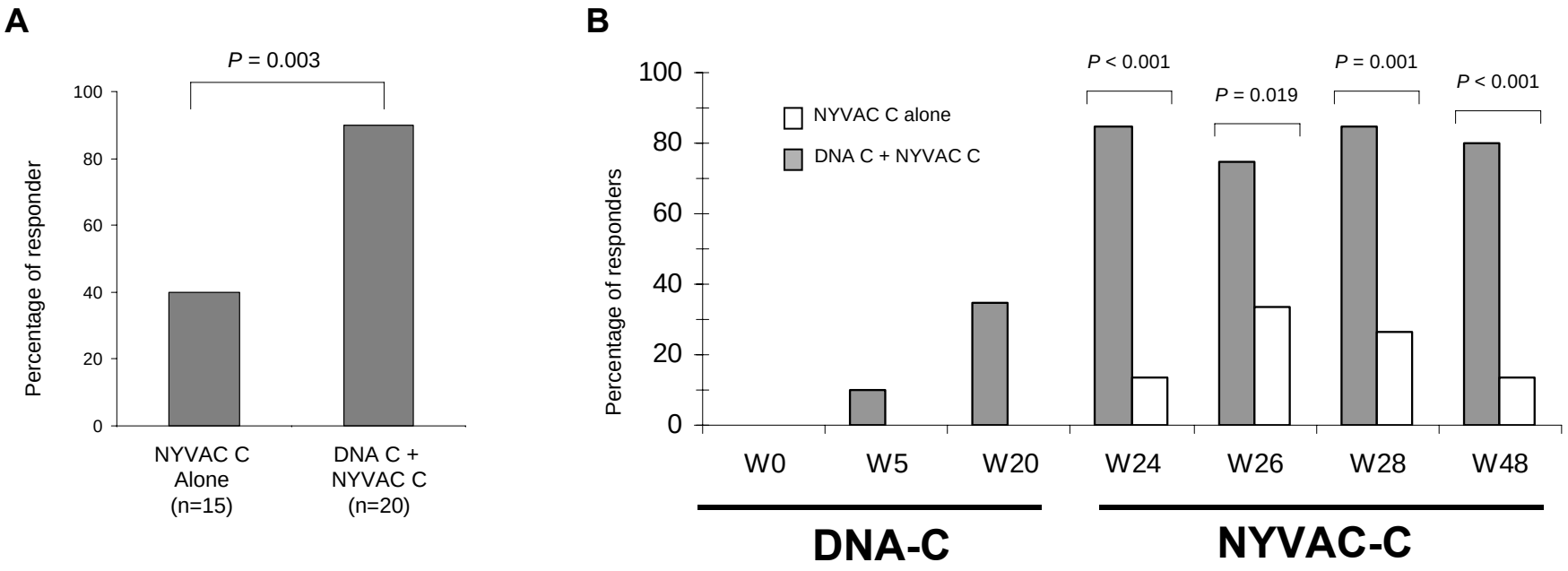
Study design:

2 x 20 HIV negative volunteers
A. DNA-C prime (W0, W4; 4mg im), NYVAC-C boost (W20, W24; 2x10E7 im)
B. NYVAC-C (W20, W24; 2x10E7 im)
London (MRC; J.Weber, S. McCormack), Lausanne (CHUV; G. Pantaleo)

Readout (Lausanne):

Several time points
ELISPOT analysis (γIFN, IL2)
FACS analysis (CD4, CD8, CCR7, IL2, γIFN)
T cell phenotype / function
Epitope mapping

EV02: Percentage of Responders (γIFN+ T cells)

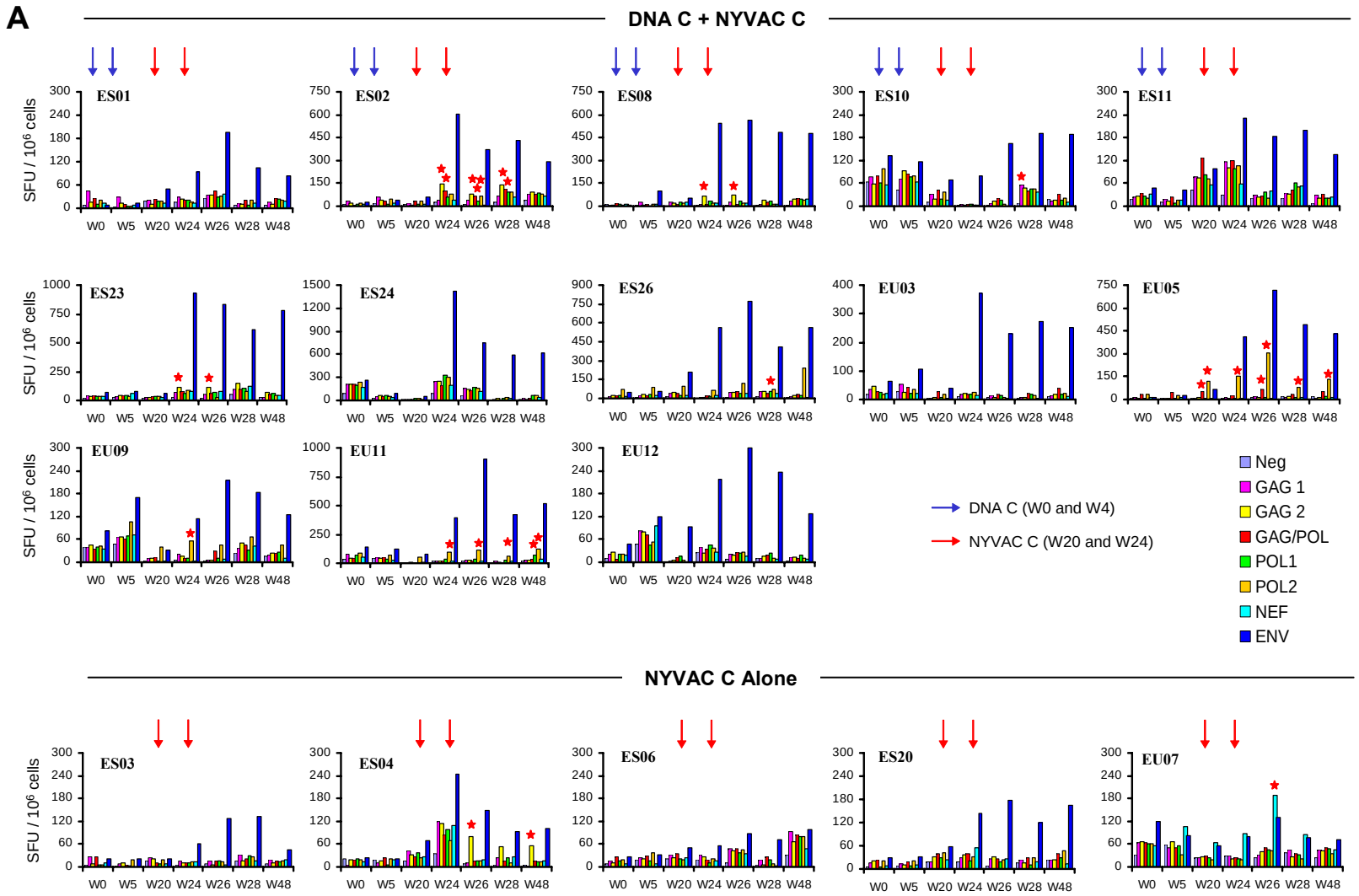


Higher percentage of responders in DNA-C prime / NYVAC-C boost group (>90%) compared to NYVAC-C group (<40%)

Durability DNA-C / NYVAC-C >> NYVAC-C

A. Harrari, G.Pantaleo *et al.*

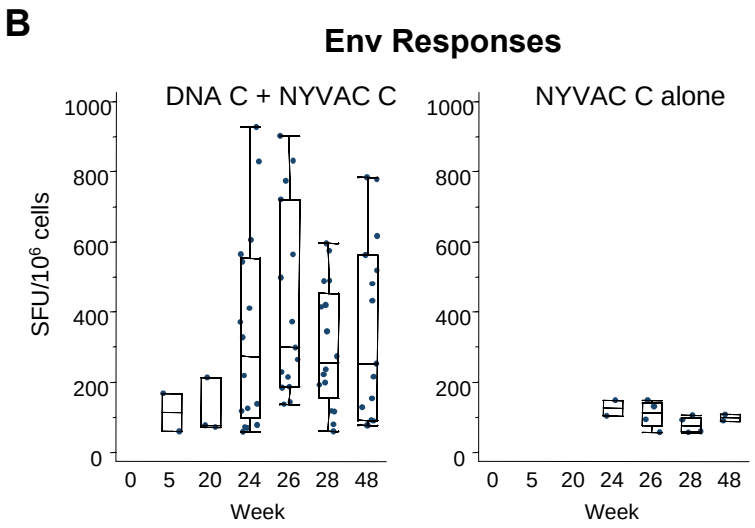
EV02: Magnitude of T cell Responses (γIFN)

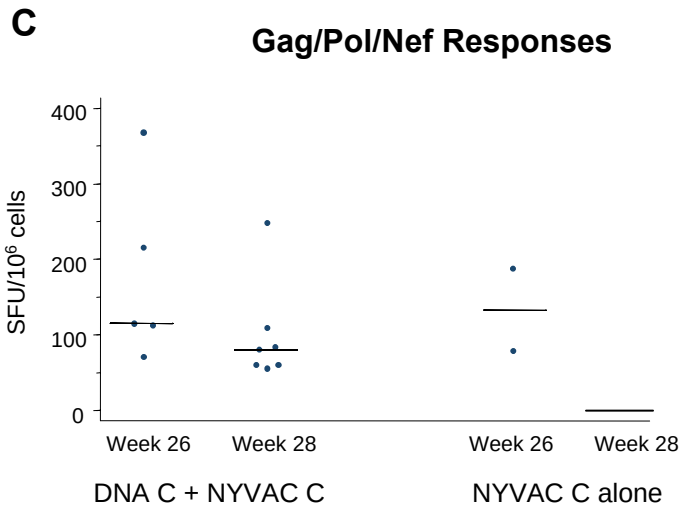


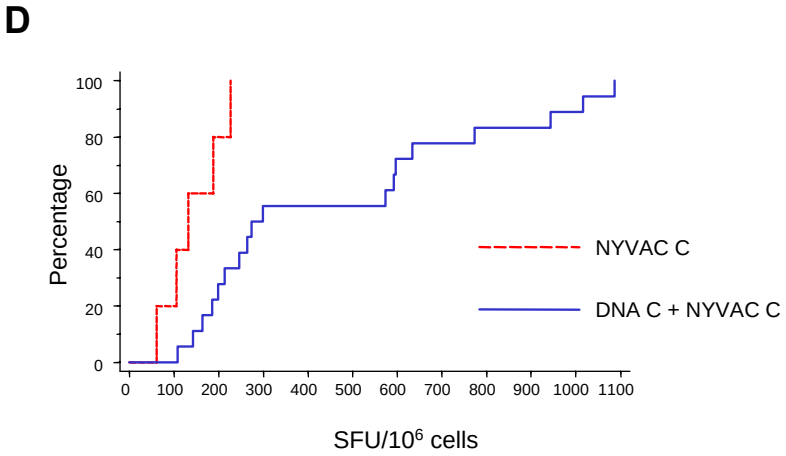
Phase I Clinical Trial

A. Harrari, G.Pantaleo *et al.*

EV02: Magnitude of T cell Responses (γIFN)



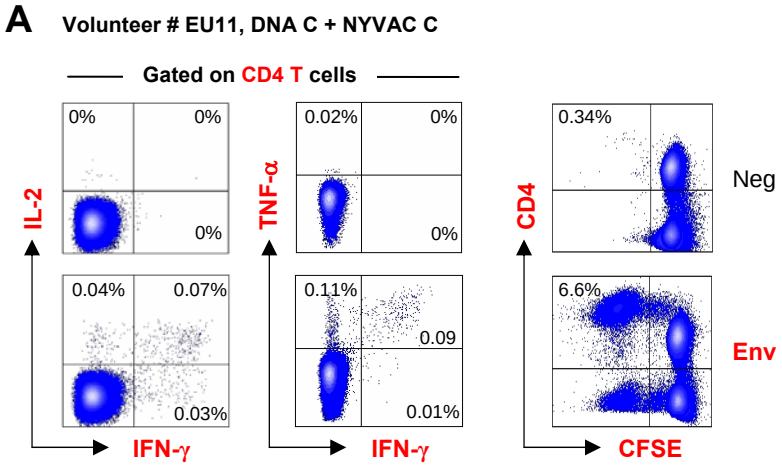




Higher magnitude of T cell reponses in DNA-C prime / NYVAC-C boost group (>90%) compared to NYVAC-C group (<40%)

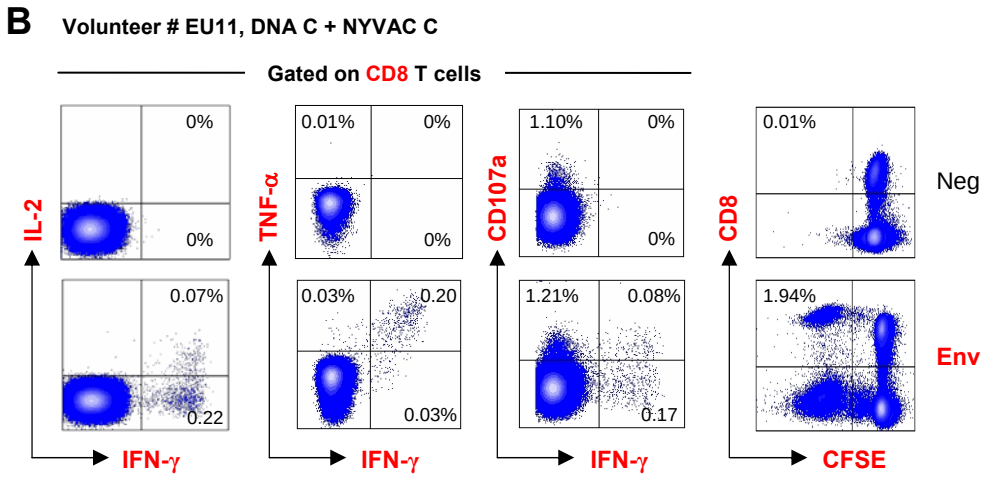
Env >> GagPolNef

EV02: Functional Analysis of T cell Responses



CD4 and CD8 responses are polyfunctional

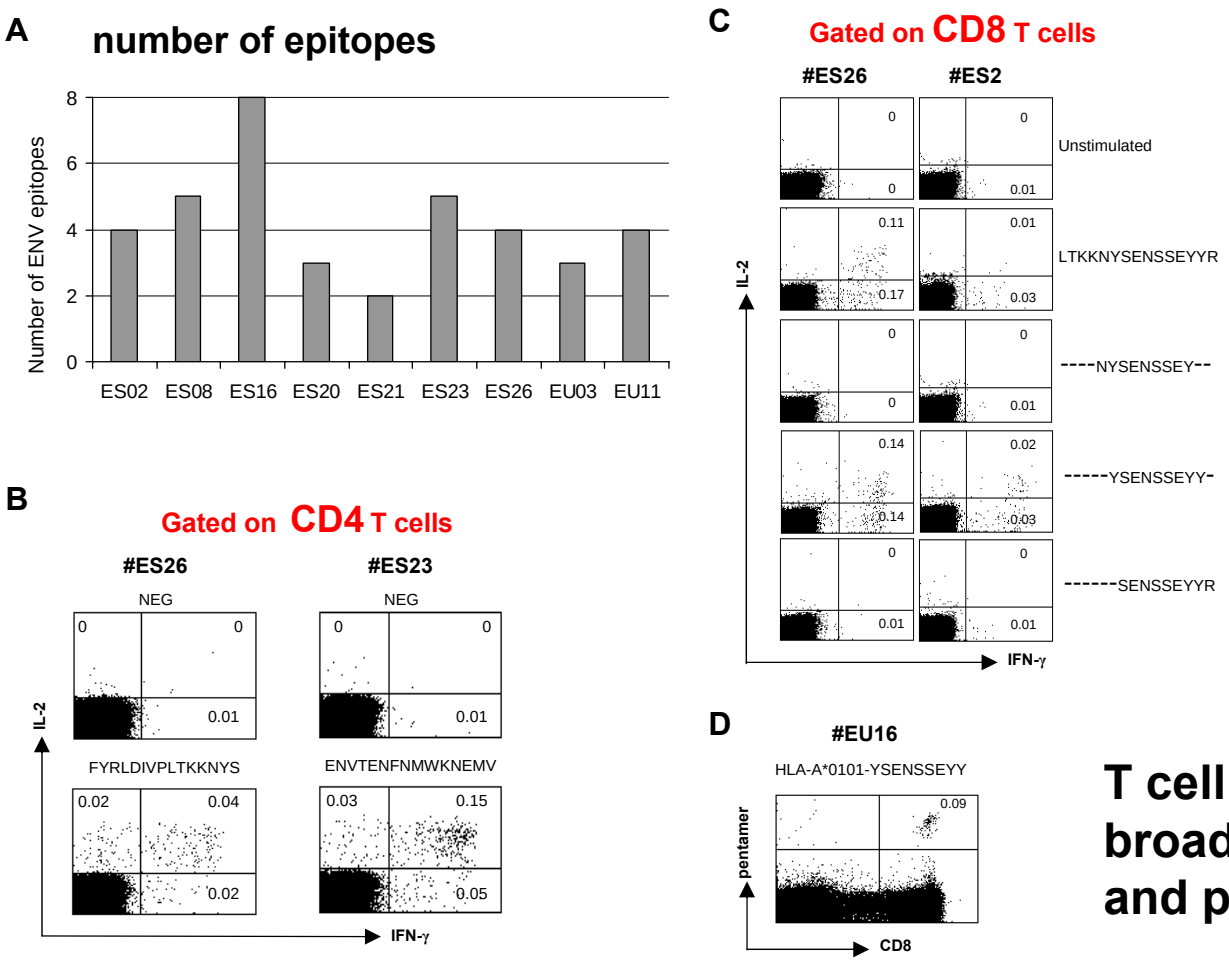
(IL-2, γIFN, TNFα)



Phase I Clinical Trial

A. Harrari, G.Pantaleo *et al.*

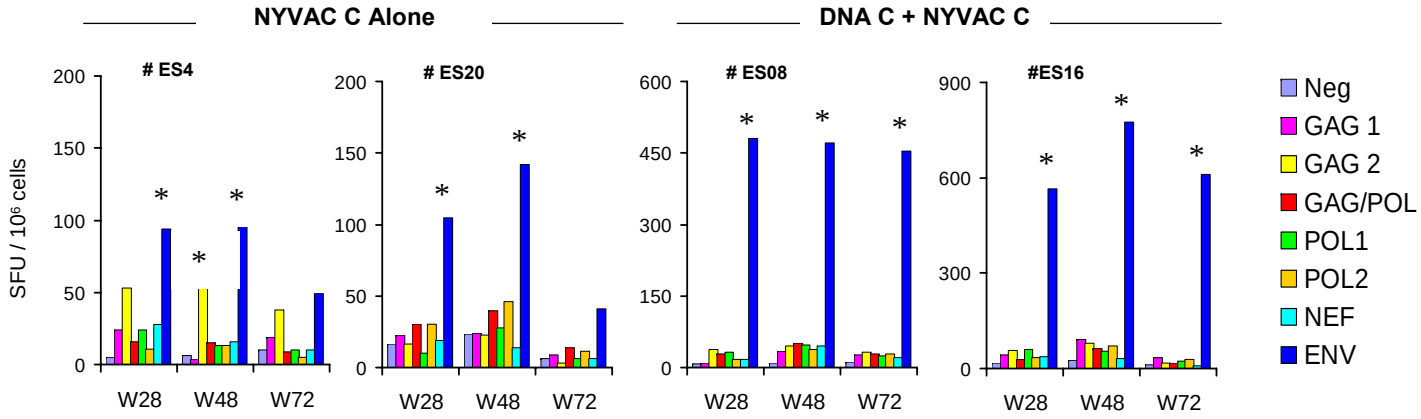
EV02: Epitope specific T cell Responses



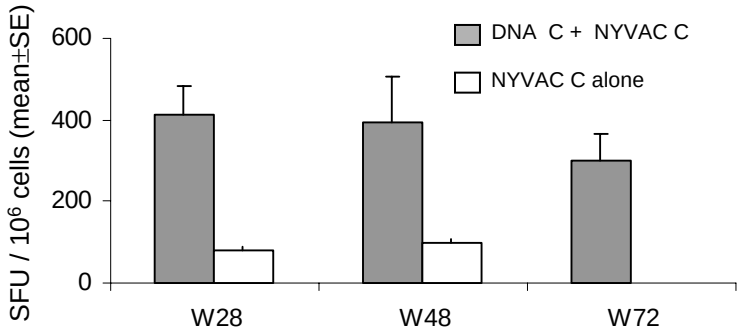
T cell responses are broad (mean 4,2 epitopes) and polyfunctional

EV02: Longevity of T cell Responses (γIFN, IL2)

A



B



Responses are durable

70% positivity at W72 (DNA-C / NYVAC-C)

~300 SFU / 10E6 cells at W72

A. Harrari, G.Pantaleo *et al.*

Dominant Env- / Mitigated Gag Responses

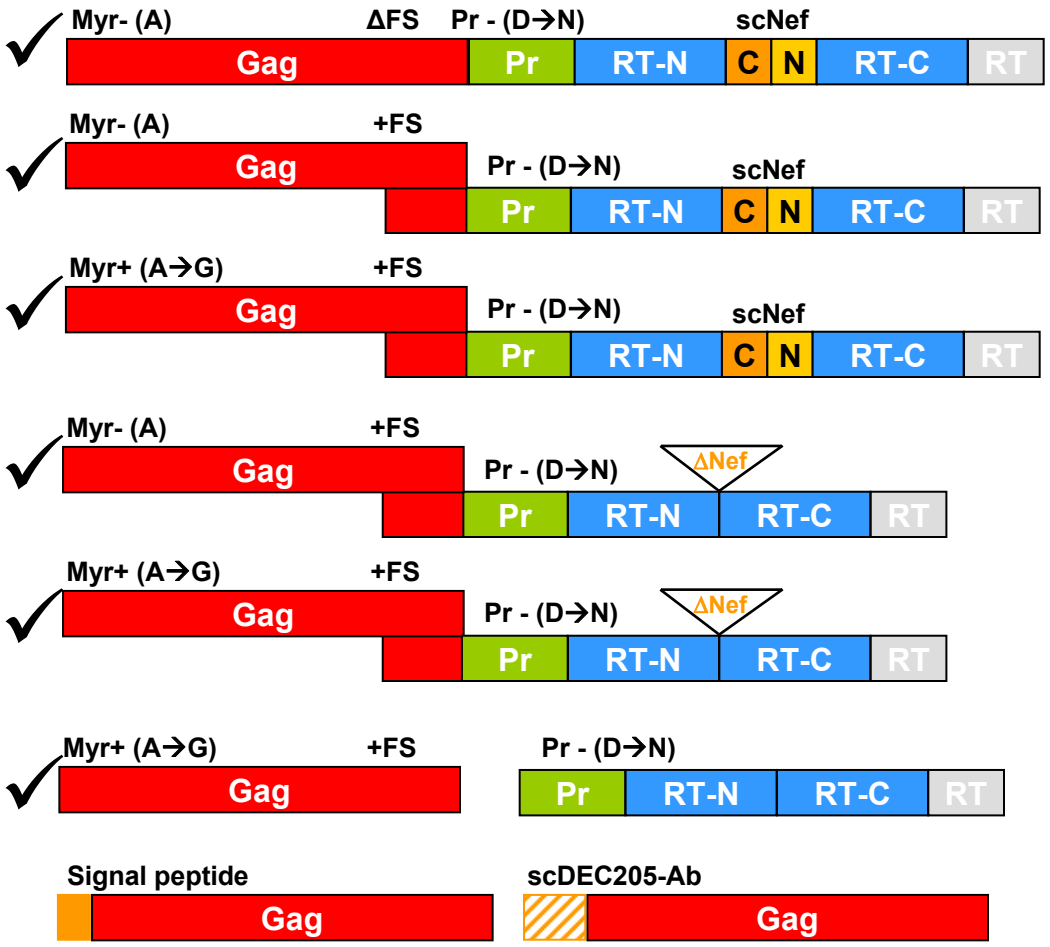
Possible explanations:

- Intracellular trafficking / localisation: gp120 secreted, GagPolNef not
- Gp120: 55 kDa + glycosylation; GPN: 160 kDa non glycosylated
- Gag expression >>> GagPolNef; SIV-Gag >> HIV-Gag
- Env vs GagPolNef: competition for HLA epitope presentation

Modifications:

- Altered immunization regimen: 3 x DNA-C (mixture DNA-GPN and DNA-Env) followed by 1 x NYVAC-C (EV03) instead 2 x DNA-C followed 2 x NYVAC-C (EV02)
- At least 2 different NYVAC viruses: Env + GagPolNef instead Env / GagPolNef; different inoculation sites
- Increase immunogen processing and epitope presentation ➔ DRIP
- Improve cross-presentation: VLP / secreted Gag derivatives
- Targeting to DCs (e.g. Dec205 targeting, FcR targeting)
- Incorporate molecular adjuvants (CD40L, CTB...)

Outlook: BMGF-PTVDC Discovery Group



reference construct

allow ribosomal frameshifting (Gag 95 / PolNef 5)

allow ribosomal frameshifting (Gag 95 / PolNef 5), VLP budding

allow ribosomal frameshifting No NEF, (Gag 95 / Pol 5)

allow ribosomal frameshifting No NEF, VLP budding

2 distinct ORFs

secretion, DC targeting

➔ **best in class: increase expression & improve access to DRIP pathway**

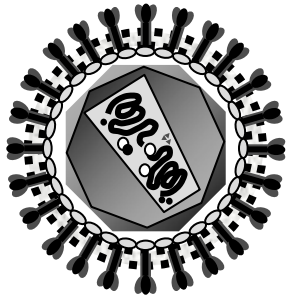
What we have achieved – Where we need to go

HIV Clades

- A (Africa)
- B (Europe, N & S-America)
- **C (Asia, Africa …)**
- E (Asia)
- F (Africa)
- G (South America, Africa)

+

Targets of immune response



- envelope proteins: **Env**
- structural proteins: **Gag, Pol**
- regulatory proteins: Tat, Rev, **Nef**
- accessory proteins: Vpr

+

Immune response

- neutralizing AB
- mucosal immunity
- **CD4+ T cell response**
- **CD8+ CTLs**
- **broad, cross clade,**
- **high quality, polyfunctional**
- **long term memory**

immunogen design
molecular virology

Delivery / Combination of Multivalent HIV/AIDS Vaccine Strategies

?

basic immunology / mice
preclinic / rhesus macaques
clinic / phase 1/2

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HIVRAD

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Bavarian Res. Foundation
Hektor Foundation

GENEART
THE GENE OF YOUR CHOICE

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