New Vaccine Strategies using cytomegalovirus

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Traditional vaccines work by mimicking the immune responses elicited by a given pathogen, using a safe alternative of the pathogen.

However, traditional vaccine approaches struggle to elicit protective immune responses for infectious diseases that do not elicit protective immunity upon natural exposure

Prophylactic Vaccines Needed

- AIDS
- Tuberculosis
- Malaria
- Hepatitis C
- Herpesviruses

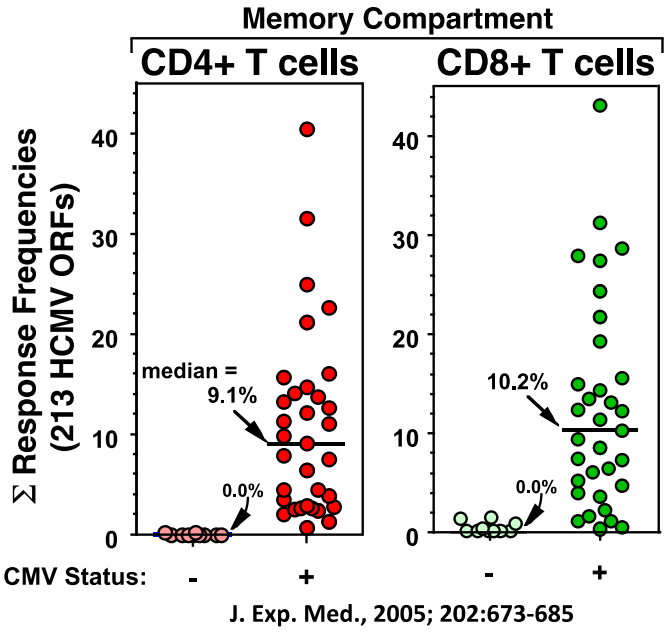
Therapeutic Vaccines Needed

- Hepatitis B
- Human papillomavirus
- Herpesviruses
- Cancer

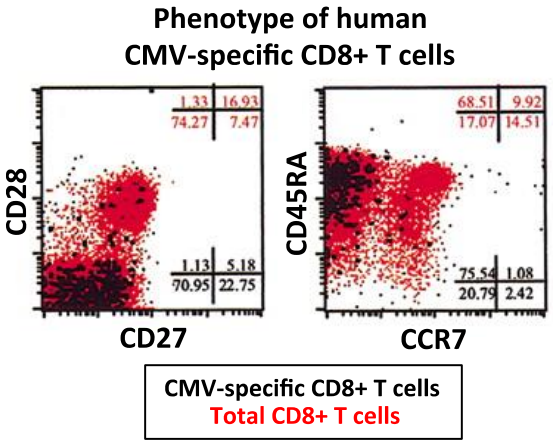
Since we cannot mimic natural immunity, we need new approaches that elicit immune responses that are different and more efficacious than those induced by each pathogen

Using Cytomegalovirus (CMV) as a vaccine vector

In the 1990s, it became evident that CMV is unique in its ability to elicit and **maintain life-long, high frequency, effector (and tissue-resident) memory T cell responses**



J. Exp. Med., 2005; 202:673-685



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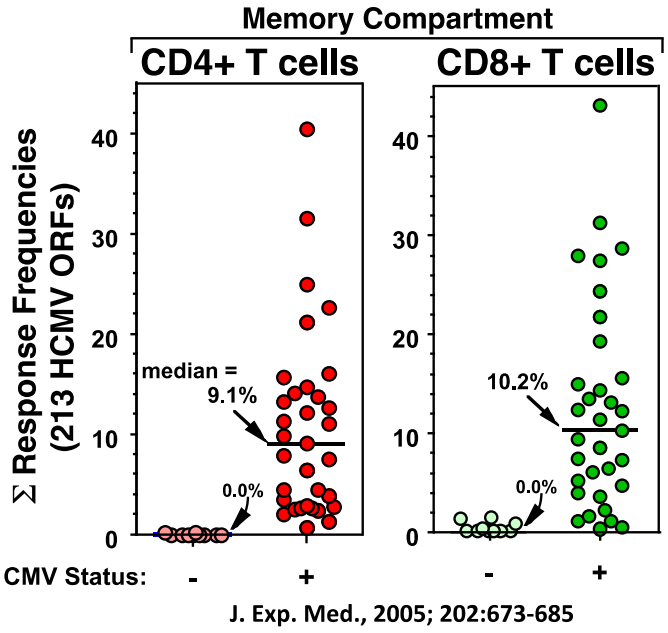
Can we use this unique immunological trait to target other pathogens?



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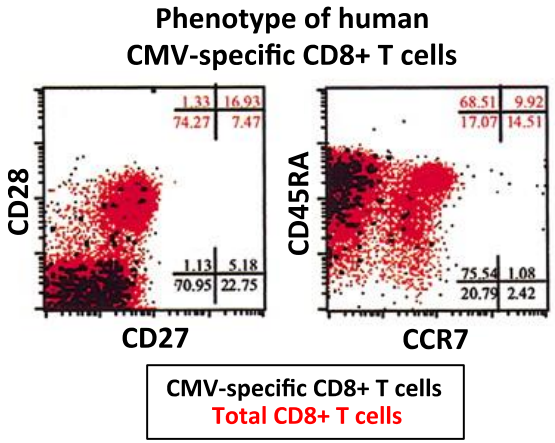

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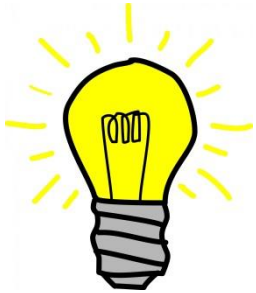

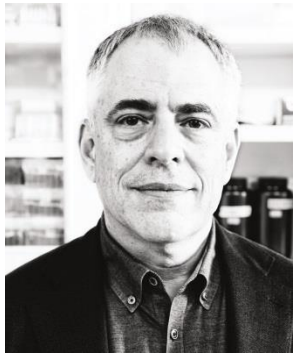

Louis Picker

Not only is the answer to this question "yes", but the immunobiologic properties of CMV vectors turned out to be markedly different from any other known vector or non-vector vaccine modality . . .

In nonhuman primates, vectors based on rhesus cytomegalovirus:

1. Elicit and indefinitely maintain high frequency, "effector memory" T cell responses* in mucosal sites, lymphoid tissues and parenchymal organs
2. Efficiently re-infect & persist despite robust anti-CMV immunity
3. Manifest uniquely programmable CD8+ T cell immunogenicity with expanded breadth and conventional and/or novel epitope targeting.
4. Provide protection against SIV, TB and Malaria
5. Maintain immunogenicity even with profound attenuation (spread-deficiency and tropism restriction)

***Little to no antibodies**

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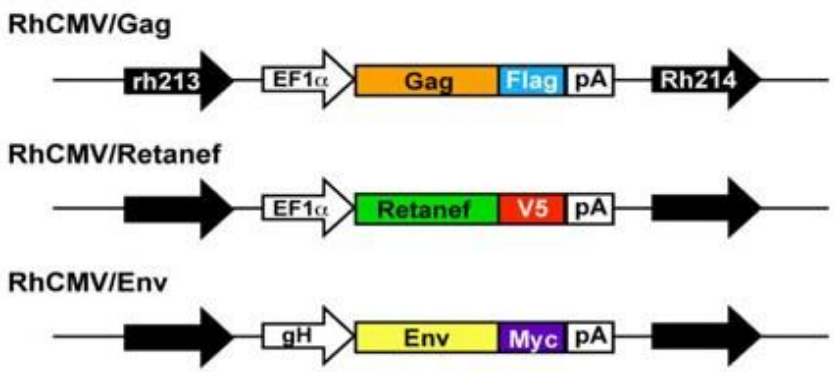
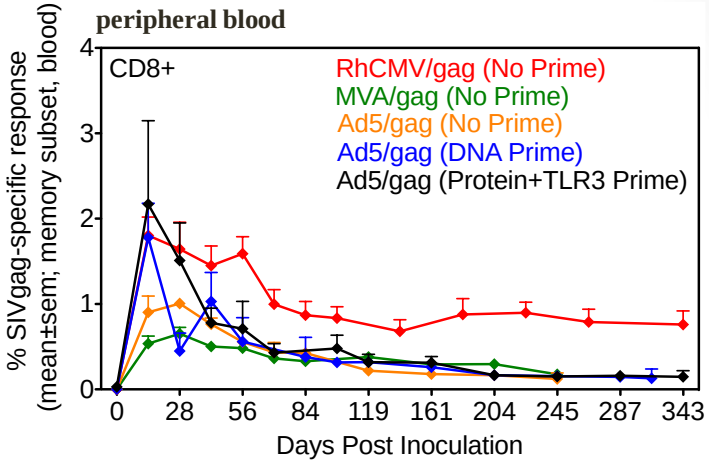
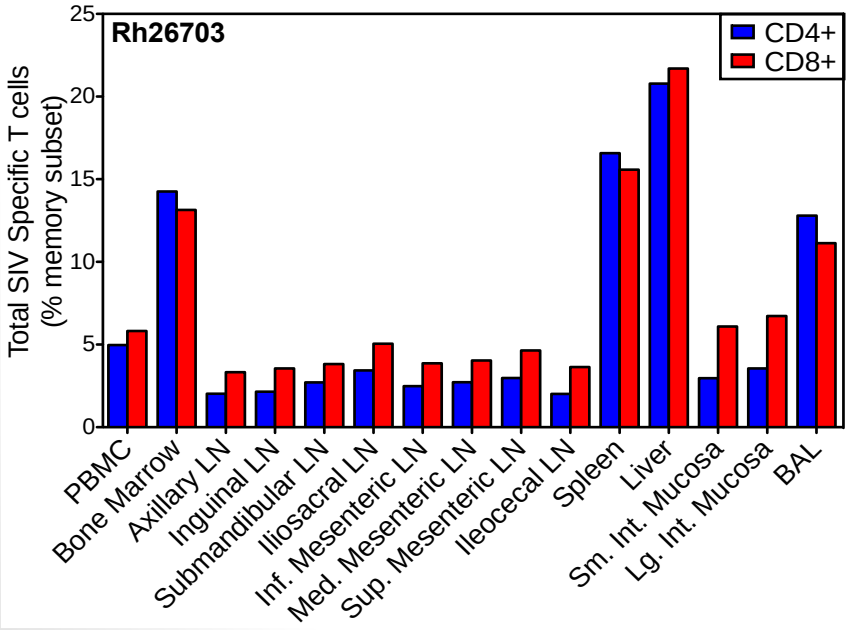
Inserting SIV antigens into RhCMV results in very high frequency SIV-specific T cells responses especially in tissues

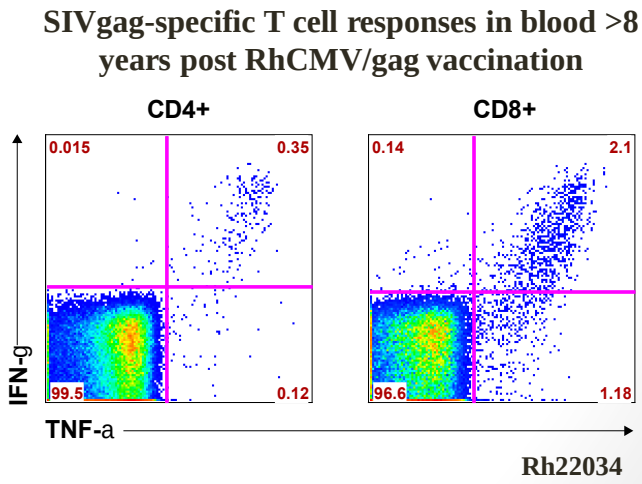




Necropsy analysis 700 days post RhCMV/SIV vector vaccination



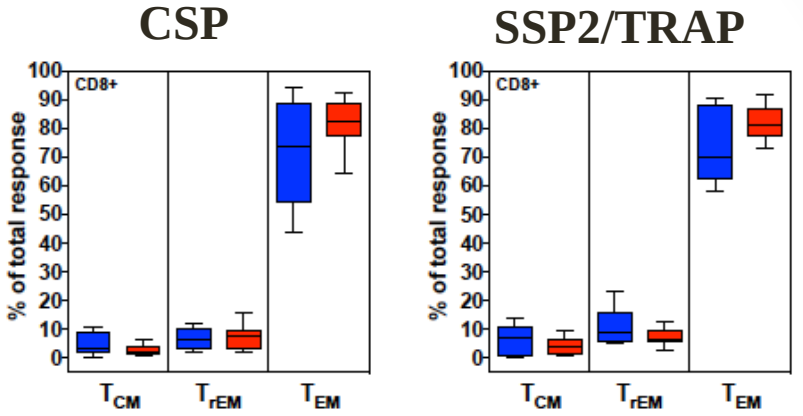
T cell responses are maintained indefinitely



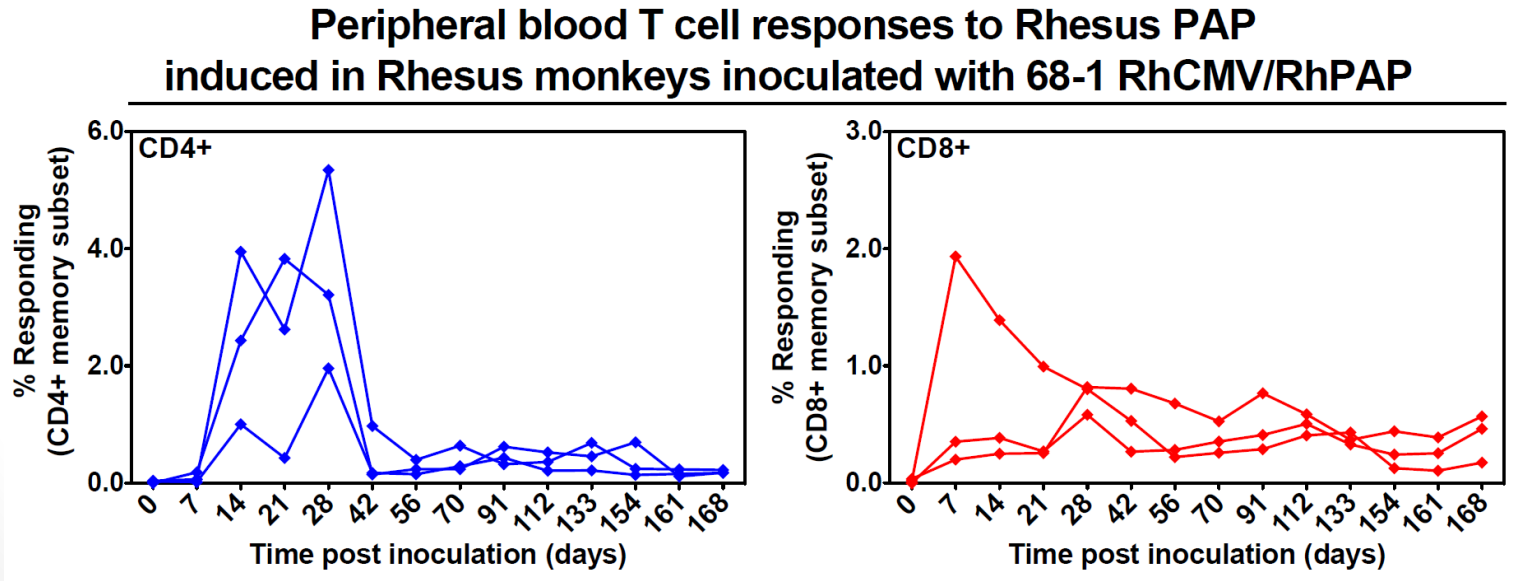
Nature Medicine, 2009

CMV vectors are able to elicit effector memory responses to many different pathogens

Mycobacterium Tuberculosis
Plasmodium knowlesi
Human Papillomavirus
Ebola Virus
Influenza Virus
Hepatitis B Virus
Herpes Simplex Virus



… even to cancer (=self) antigens



unpublished

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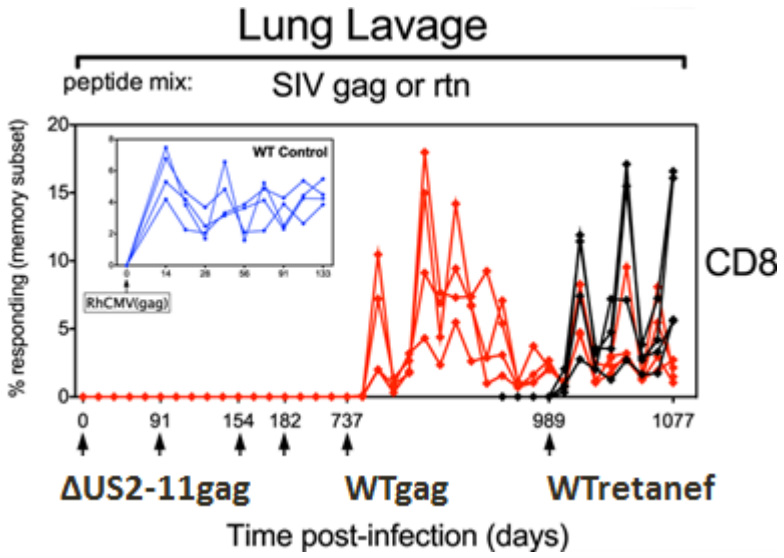
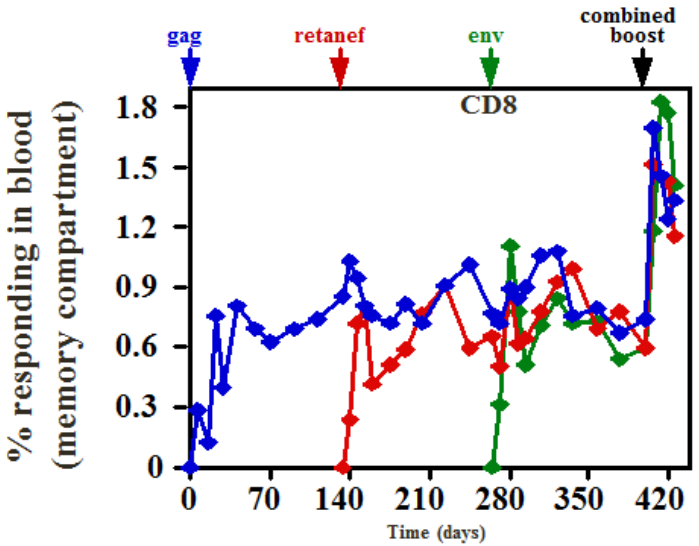
*Little to no antibodies

Viral inhibitors of antigen presentation (homologs of US2, US3, US6, US11) allow CMV-based vectors to be used repeatedly in CMV-immune recipients



Science, 2010



Nature Medicine, 2009

10 PFU sufficient for subQ reinfection!

This feature permits testing RhCMV-based vaccines in CMV-positive animals + humans

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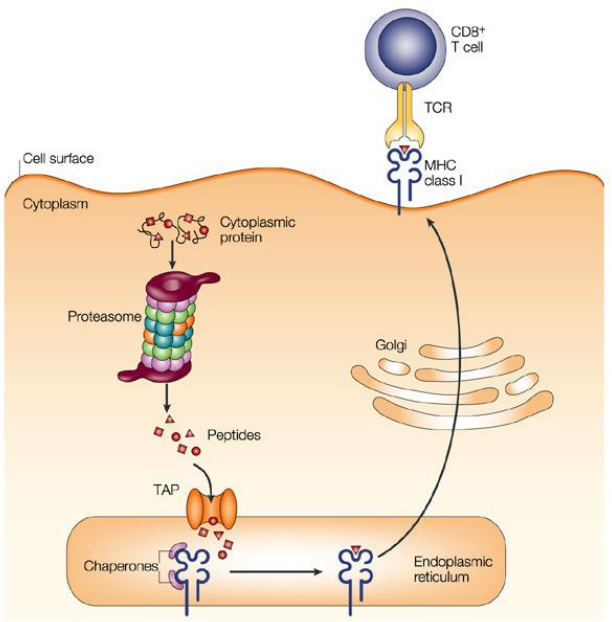
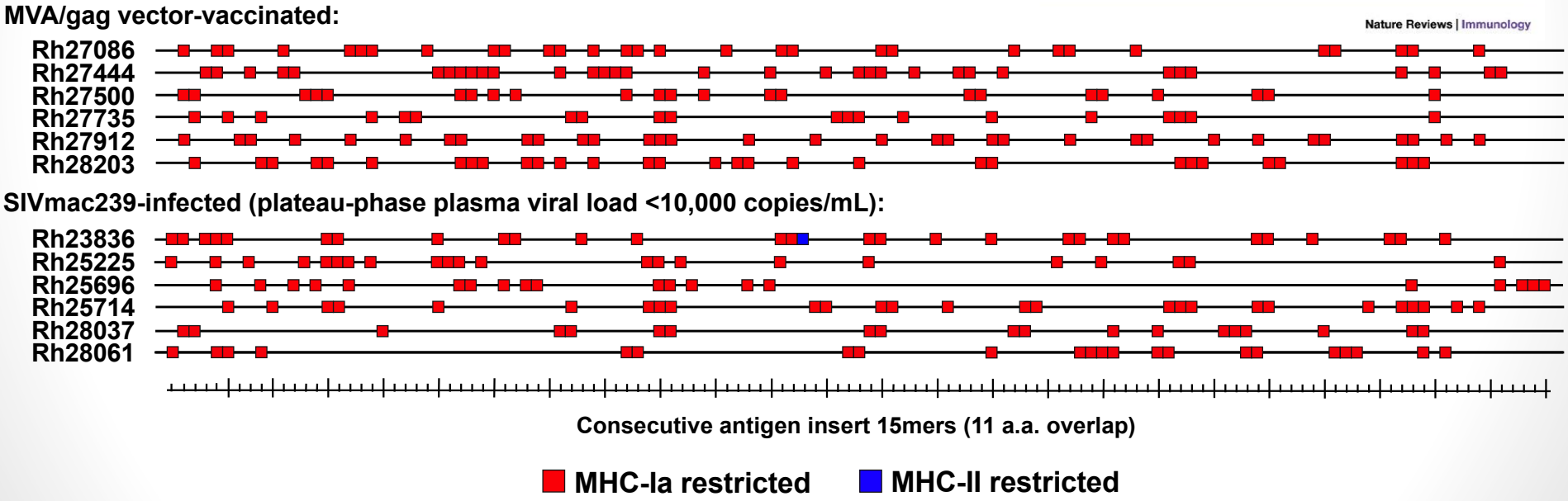
Viral infections and conventional vaccine vectors elicit CD8+ T cells that recognize peptides presented by polymorphic MHC-Ia molecules

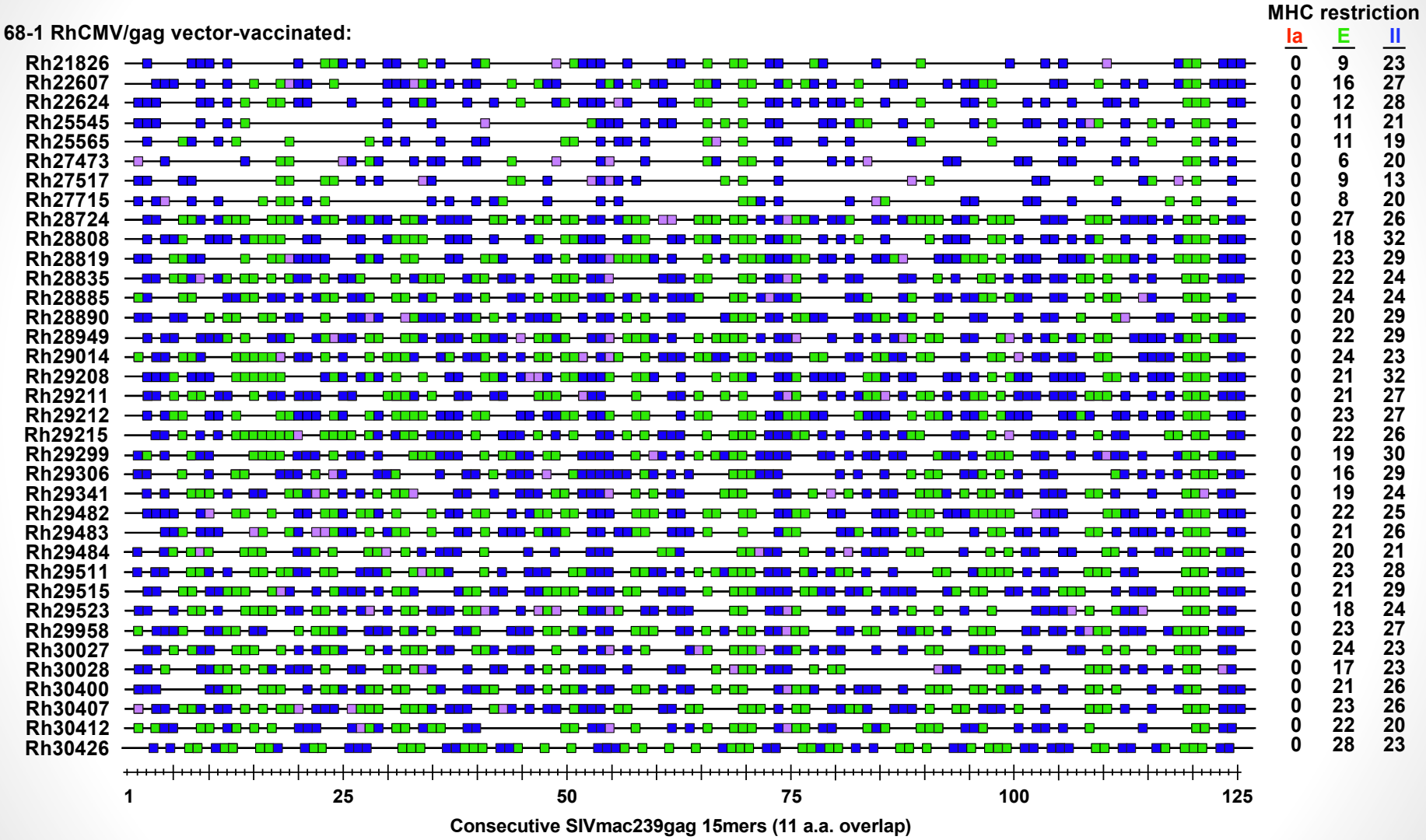




Each square represents the location of a peptide recognized by CD8+ T cells within SIVgag

The color represents the restriction element as determined by blocking antibodies or peptides

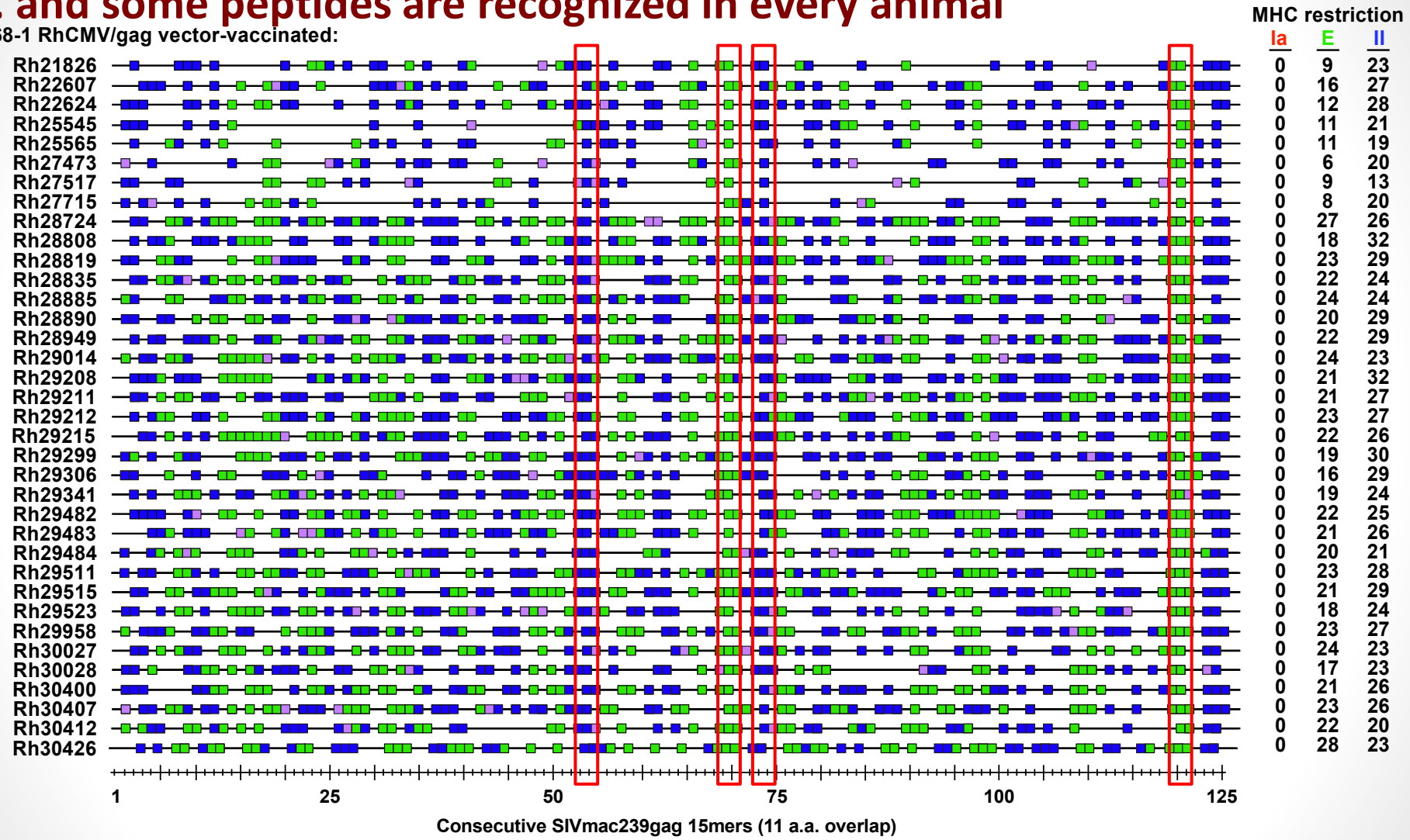
RhCMV-based vectors elicit CD8+ T cells that recognize peptides in the context MHC-II or the non-polymorphic MHC-Ib molecule MHC-E



Science 2013, 2016

RhCMV-based vectors elicit CD8+ T cells that recognize peptides in the context MHC-II or the non-polymorphic MHC-Ib molecule MHC-E

… and some peptides are recognized in every animal

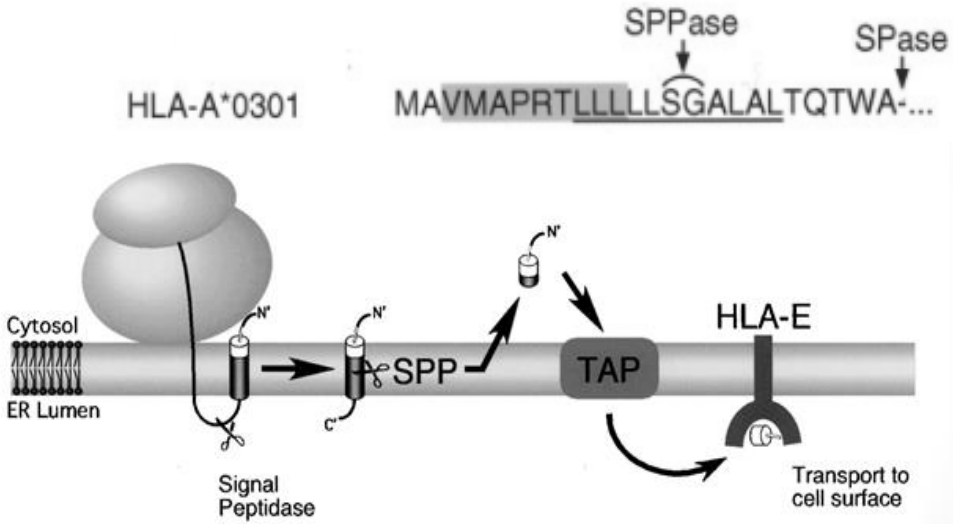


Supertopes

Science 2013, 2016

MHC-E is generally not involved in antigen presentation to T cells but presents peptide VL9 from MHC-I leader sequences as a “self” signal to NK cells

MHC-E is loaded with VL9
TAP dependent



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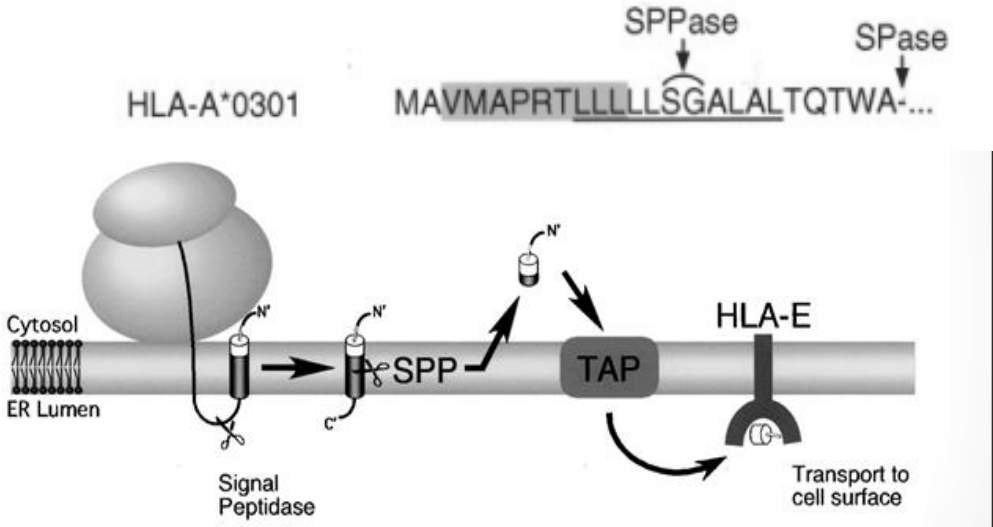
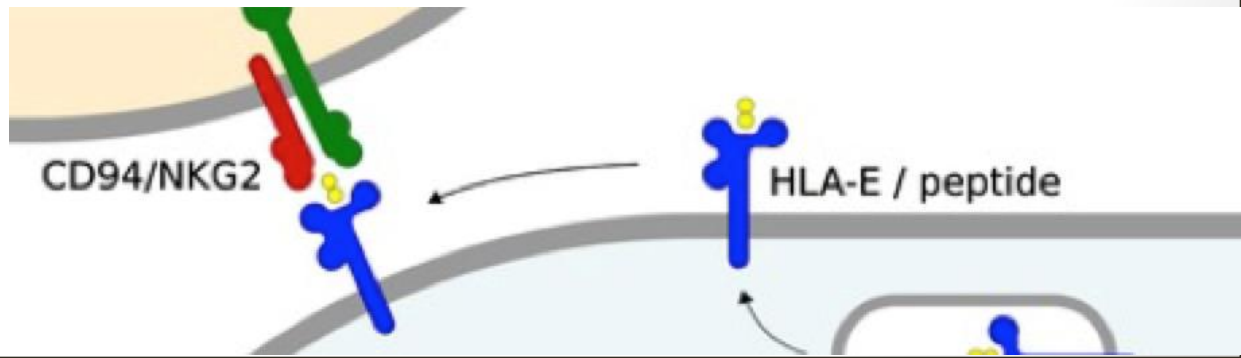
TAP dependent

MHC-E/VL9 complex represents a self-signal for NK cells

NKG2A: inhibitory receptor



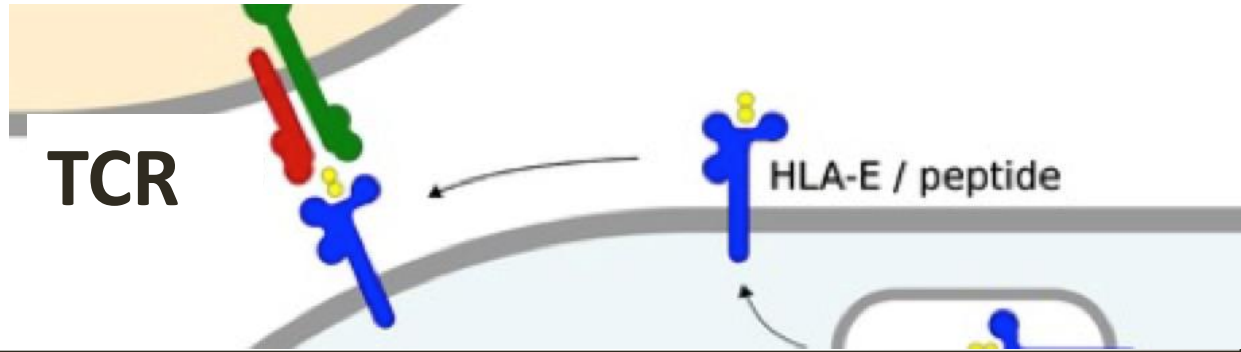


Importantly, although CMV is unique in its ability to elicit CD8+ T cells that recognize peptides in the context of MHC-E, other viruses (e.g. SIV, HIV, HBV) as well as cancer cells can be recognized by MHC-E-restricted CD8+ T cells.

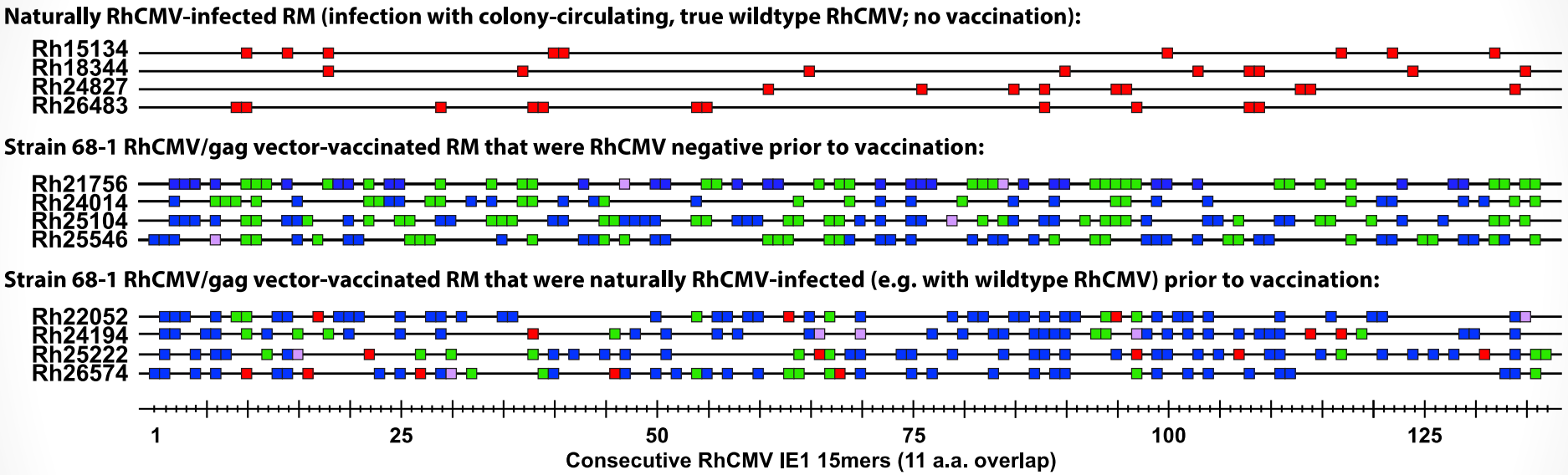
This suggests that not all of MHC-E is loaded with VL9 peptide and that non-VL9 loading (or VL9 exchange) is a common occurrence in MHC-E expressing cells.

HLA-E is upregulated by chronic pathogens (e.g. HIV, HCMV, HCV) and on many cancers

The non-polymorphic nature of HLA-E offers an entirely new vaccine modality



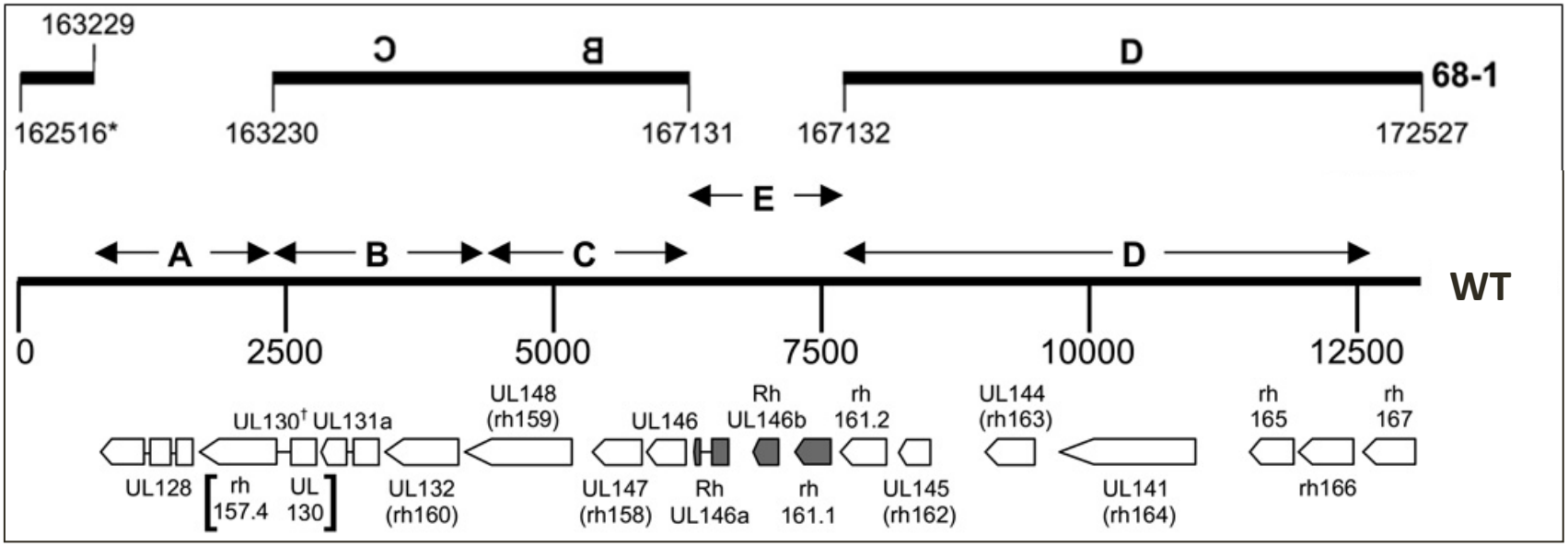
Unconventionally targeted CD8+ T cell responses (to CMV proteins) are not found in naturally CMV infected monkeys (or humans)



Only in animals given RhCMV vectors derived from strain 68-1

Science 2013, 2016

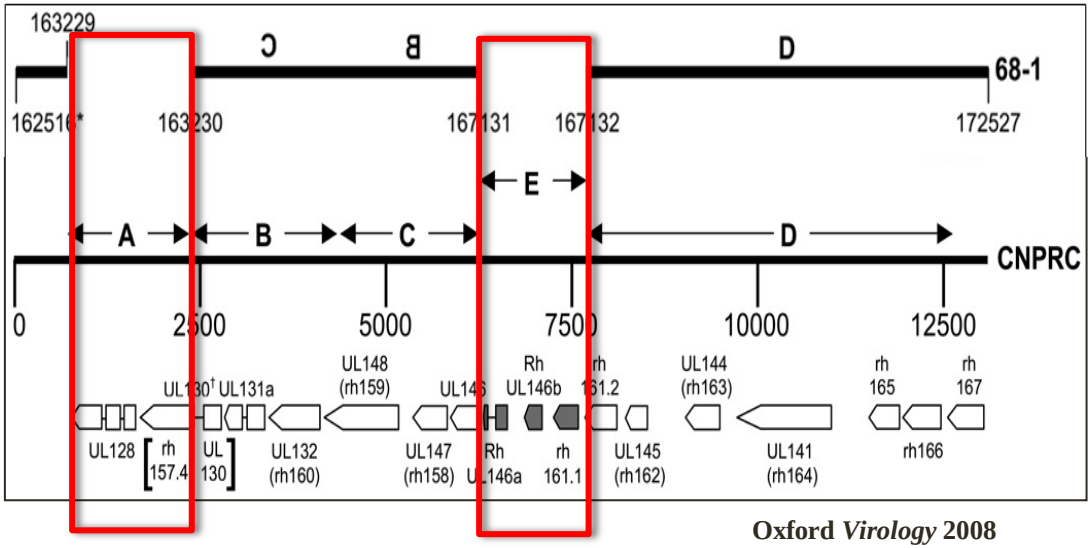
RhCMV 68-1 is a fibroblast-adapted strain with gene deletions and inversions



Oxford *Virology* 2008

Which of these spontaneous genetic modifications enable RhCMV 68-1 to elicit unconventional CD8+ T cells?

Repair of deletions switches the CD8+ T cell responses from unconventional to conventional



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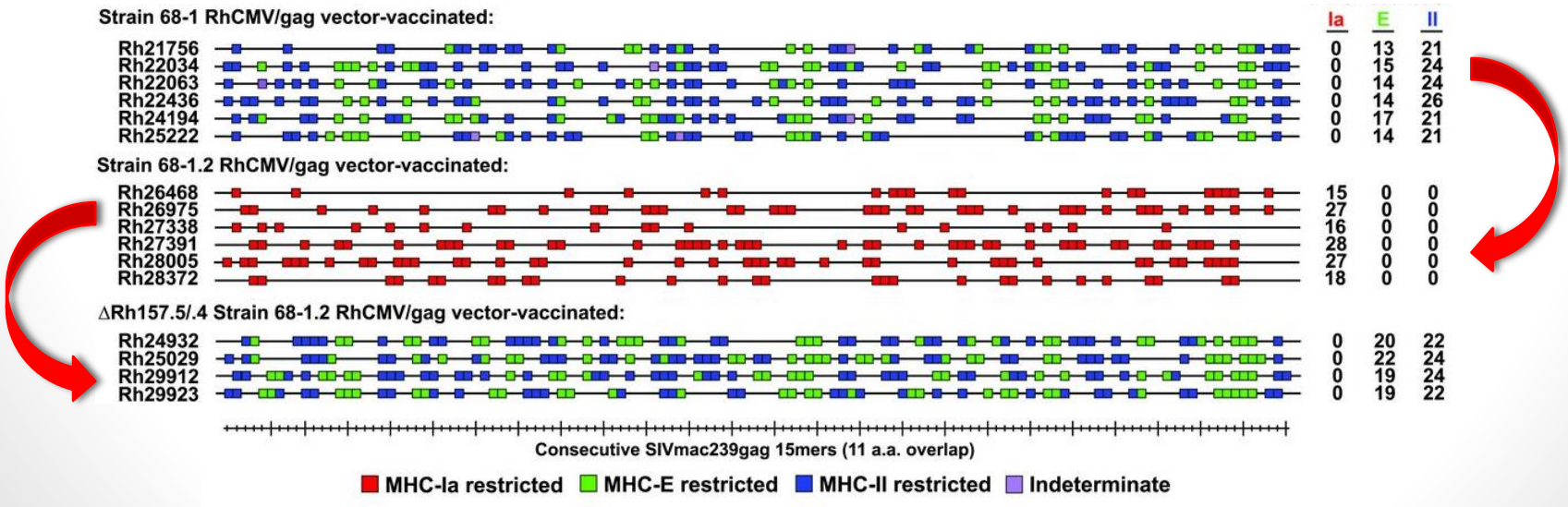
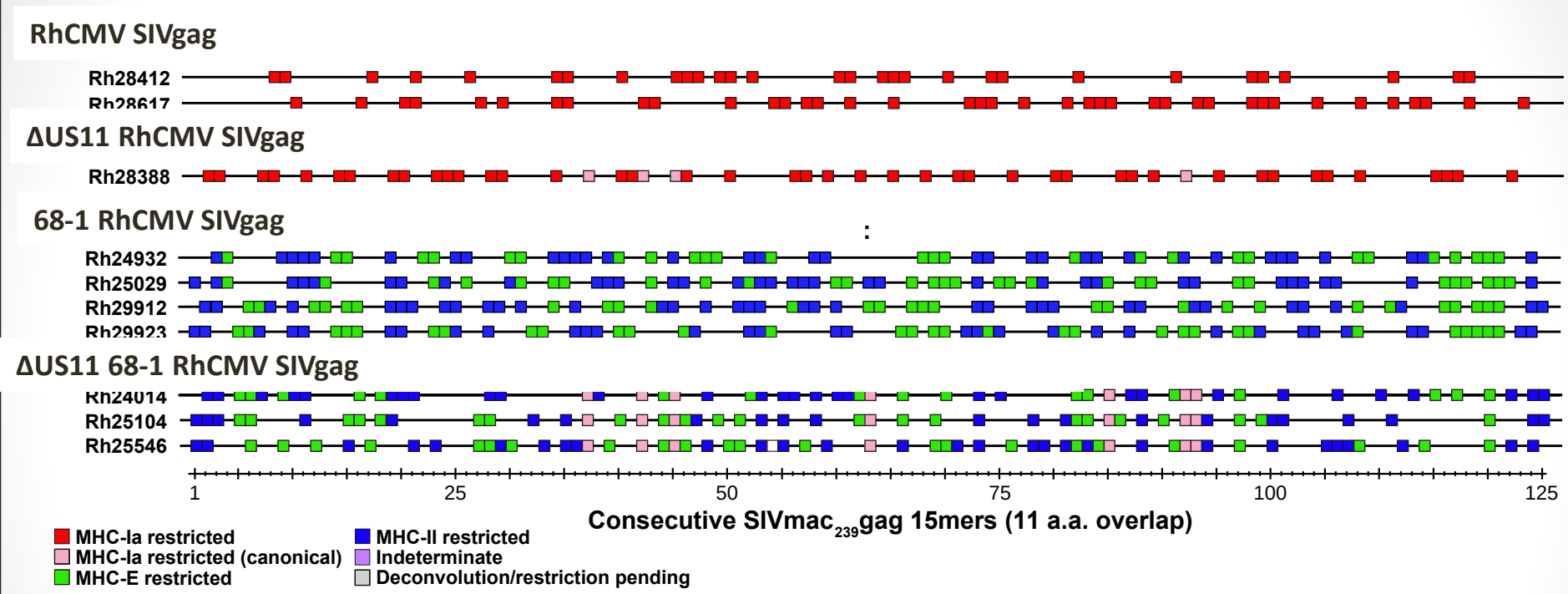


Science 2013, 2016

Deletion of US11 “programs” RhCMV to additionally induce canonical MHC-I-restricted CD8+ T cells

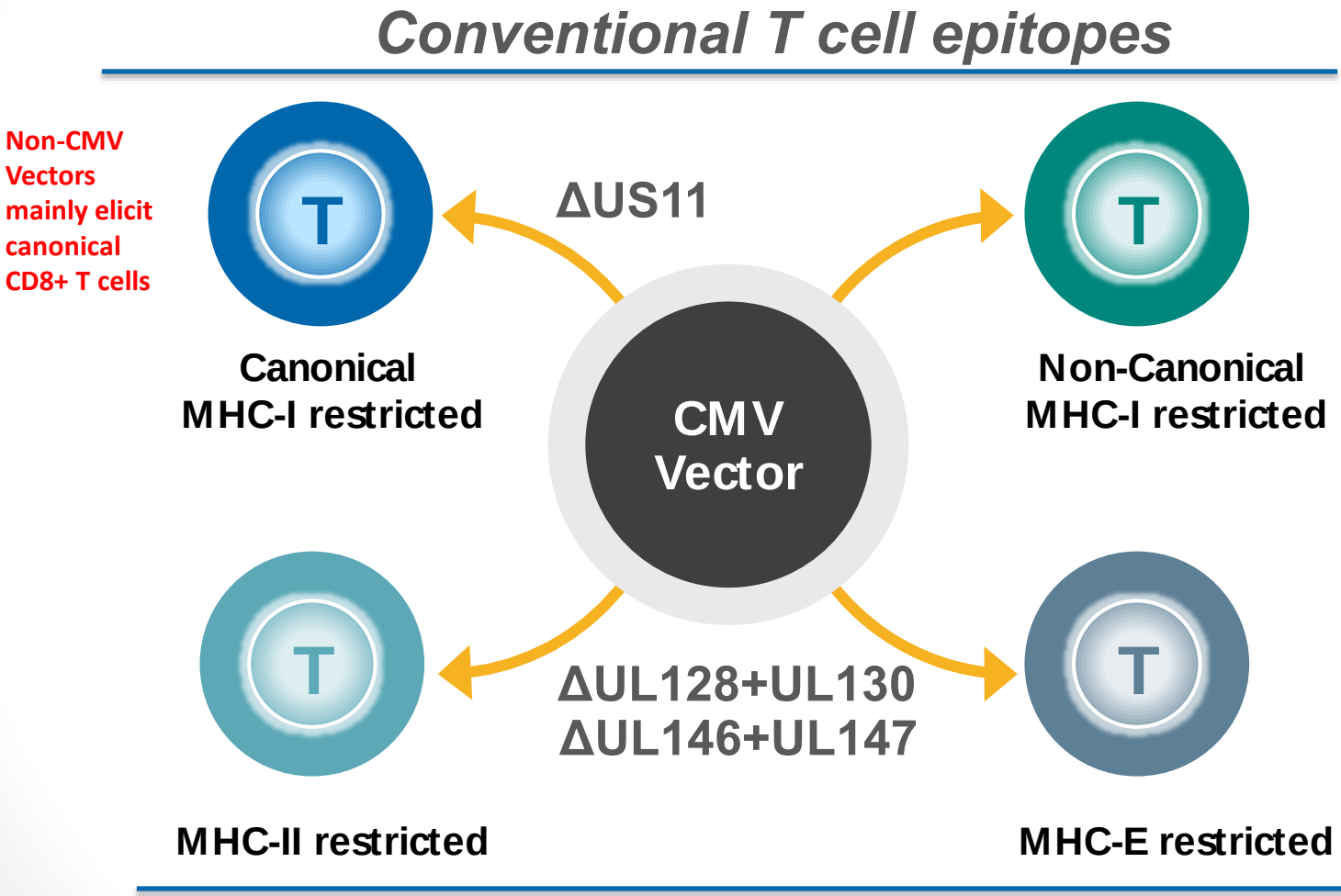


Non-canonical
Canonical

in either a conventional or unconventional response background

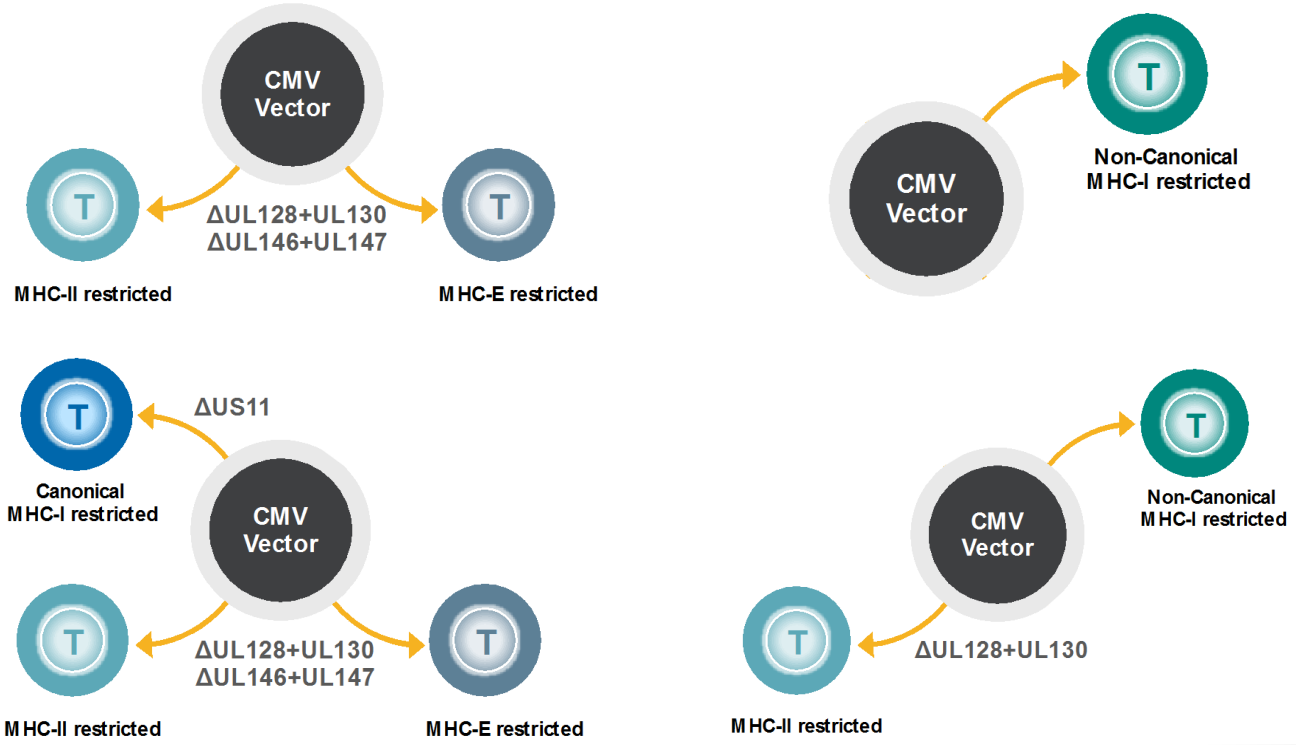
Science 2013, 2016

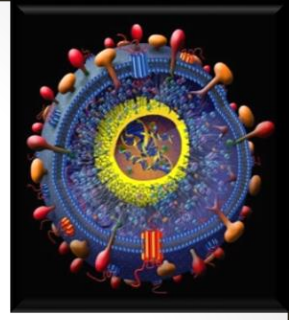
We can program four non-overlapping CD8+ T cell responses!



Current Opinion in Immunology 2017

Using different vector backbones we can now determine the role of conventional (canonical/non-canonical) and unconventional (MHC-II, MHC-E) CD8+ T cell responses in protection against pathogen challenge





SIV

SIVmac239: non-human primate AIDS model

- SIVmac239
  - Highly pathogenic in Indian origin Rhesus macaques
  - 10-100 times more virulent than HIV in humans
- Challenge model: repeated low dose intra-rectal or intra-vaginal inoculation to recapitulate natural infection with HIV

Grand Palace, Bangkok

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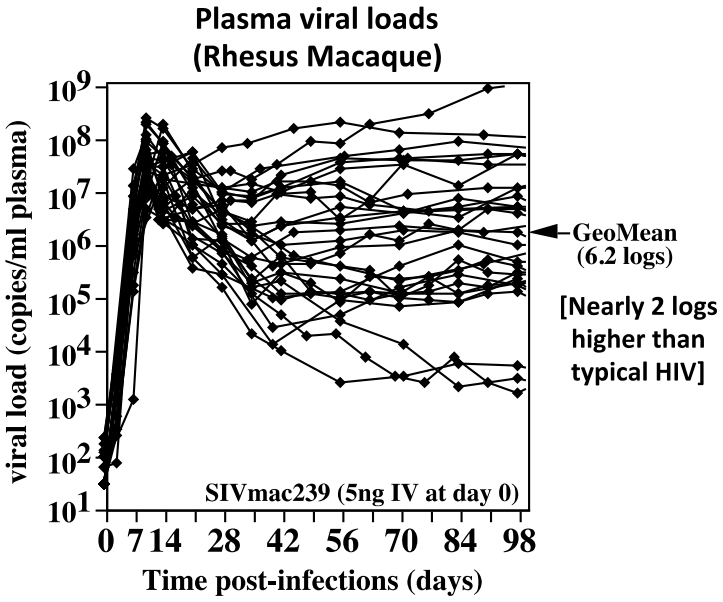
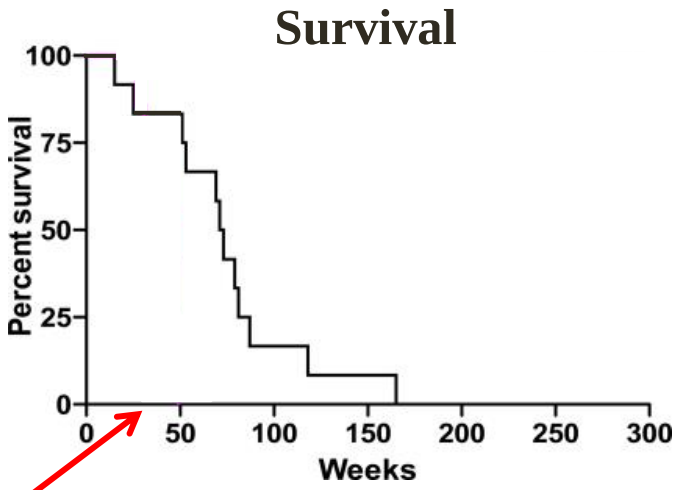
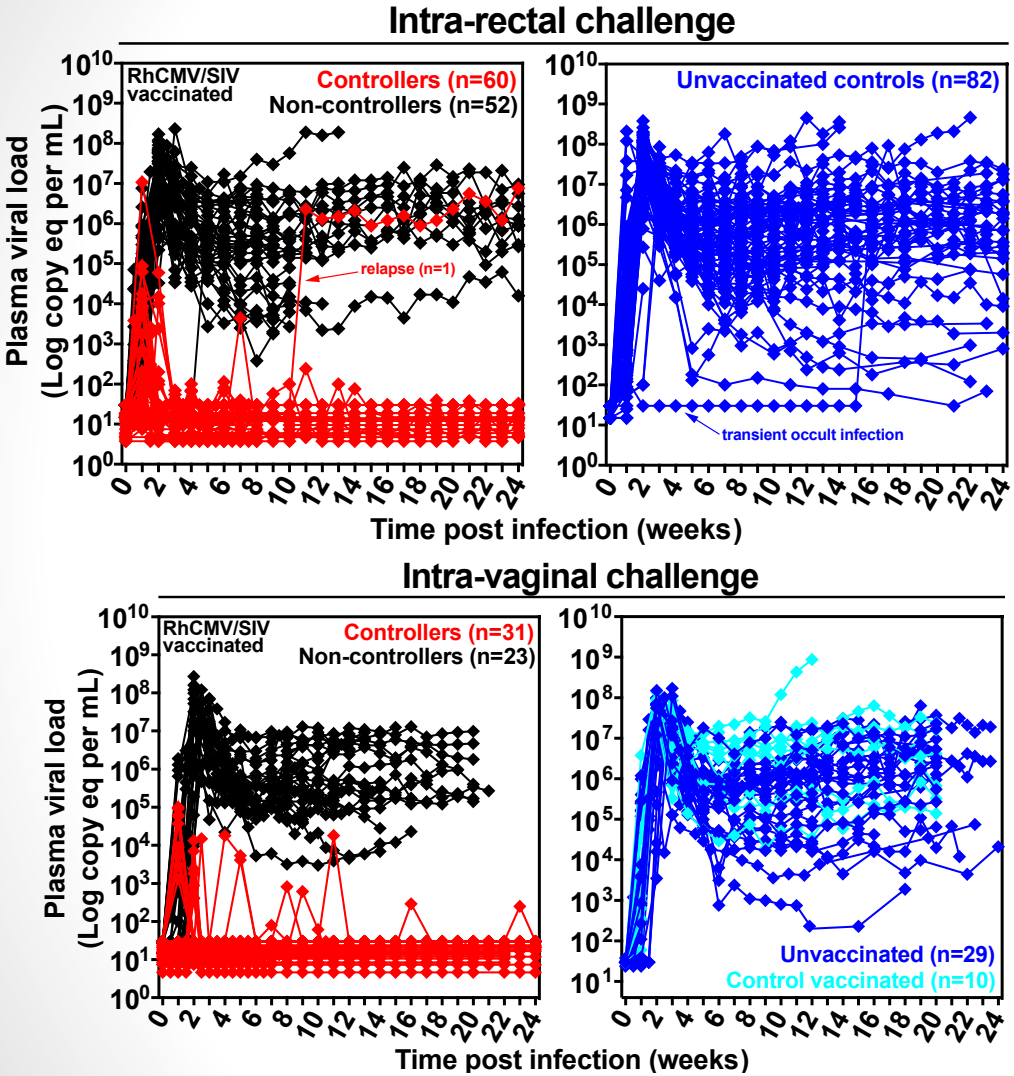


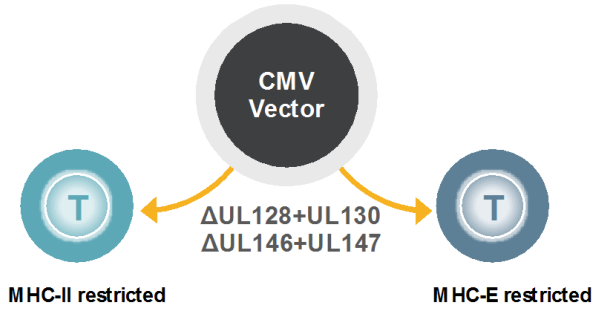


Ave. time to AIDS in SIVmac239-infected RM is ~8-fold faster than for HIV infection!

Nature, 2011

RhCMV/SIV vaccine are the first to control highly virulent SIV





Vaccine: 68-1 RhCMV Multiple vectors Expressing gag, rev, tat, Nef, pol +/- env

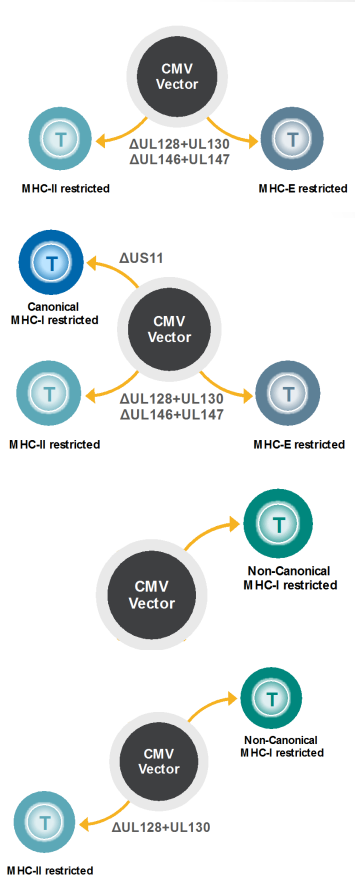
All or nothing control!

Overall efficacy = 55% (91/166 animals protected)

Nature Medicine 2009, Nature, 2011, 2013

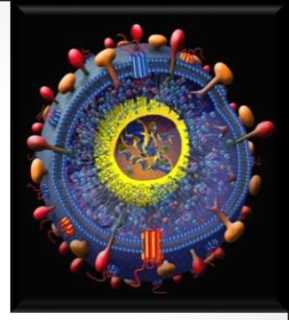
Testing different CD8+ T cell programs against SIV

- 68-1 (MHC-E + MHC-II)
  - 55% protection (91/166 animals challenged)
- 68-1ΔUS11 (MHC-I*canonical* + MHC-E + MHC-II)
  - 21 % protection (3/14 animals challenged)
- 68-1.2 (MHC-I*non-canonical*)
  - 0% protection (0/10 animals challenged)
- 68-1.2ΔUL128 (MHC-I*non-canonical* + MHC-II *non-supertopes*)
  - 0% protection (0/14 protected)



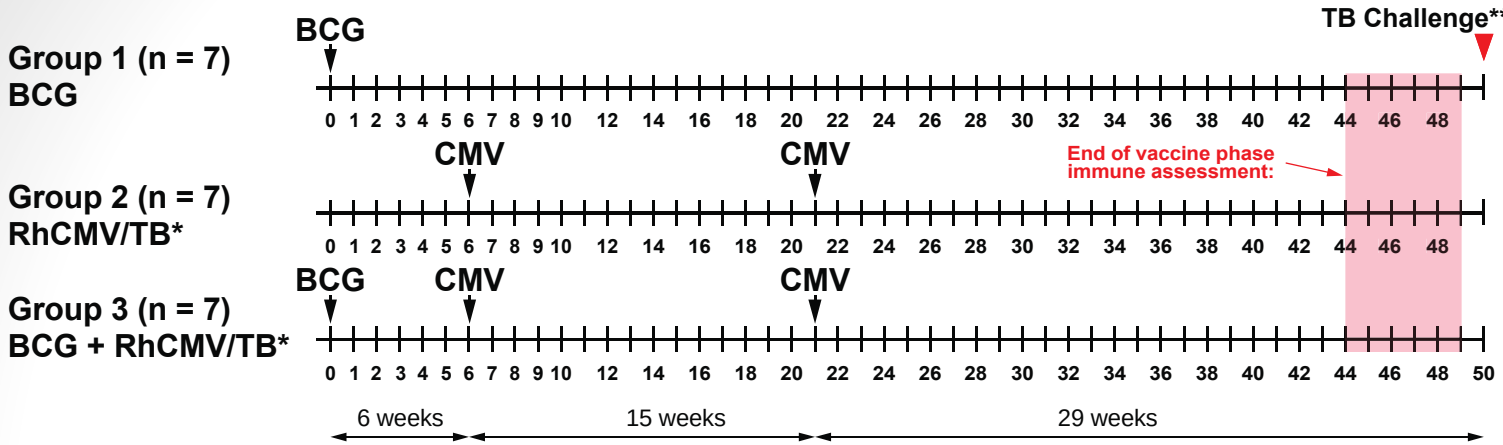
- **Current Status: Protection against SIV requires MHC-E and/or MHC-II*supertope* targeting CD8+ T cells**
  - Ongoing experiments: testing protection by CD8+ T cells that are MHC-E only, MHC-II only, MHC-Ia only (not 68-1.2), MHC-E supertopes only, MHC-II supertopes only

unpublished



TB

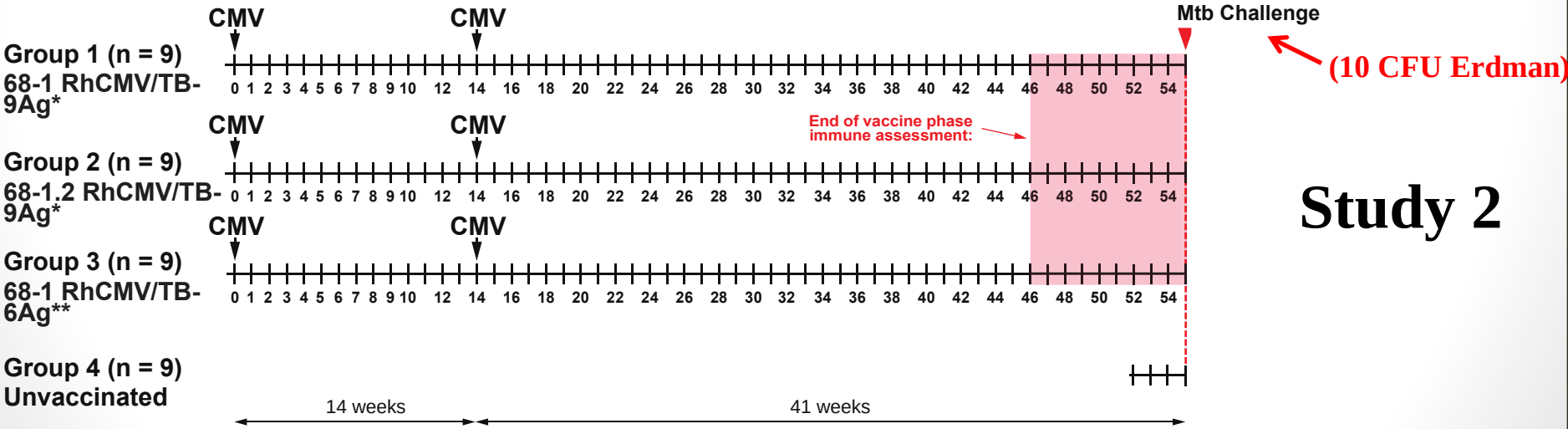
Rhesus macaques are highly susceptible to Mtb



Study 1

*68-1 RhCMV: Ag85A/Ag85B/Rv3407; Rpf A/Rpf C/Rpf D; Rv1733/Rv2626; Ag85B/ESAT-6

+ Group 4 (n = 8): unvaccinated controls

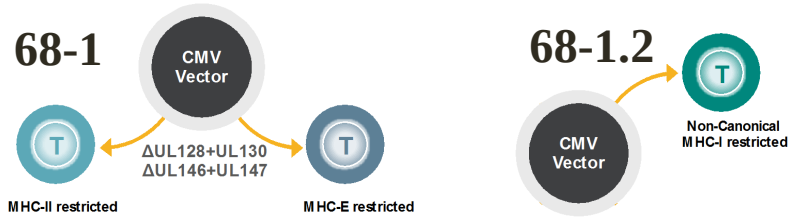


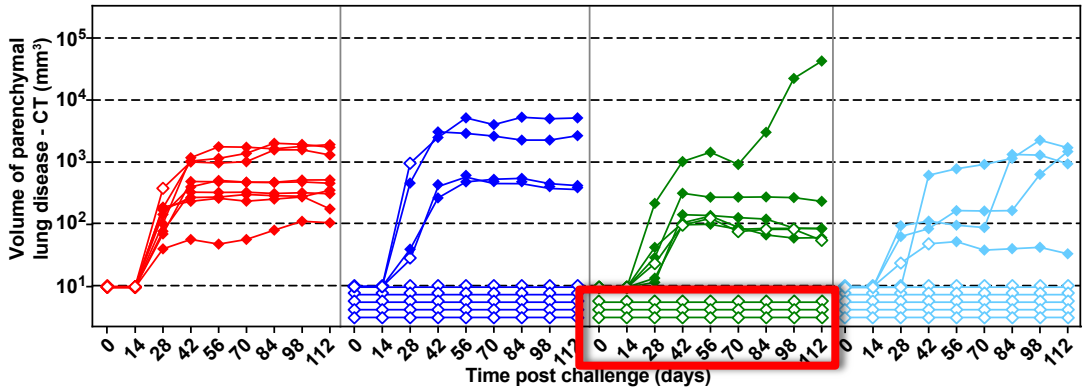
Study 2

*RhCMV(Ag85A/Ag85B/Rv3407); RhCMV(Rpf A/Rpf C/Rpf D); RhCMV(Rv1733/Rv2626); RhCMV(Ag85B/ESAT-6)
**RhCMV(Ag85A/ESAT-6/Rpf A/Rpf D/Rv2626/Rv3407)

Nature Medicine, 2018

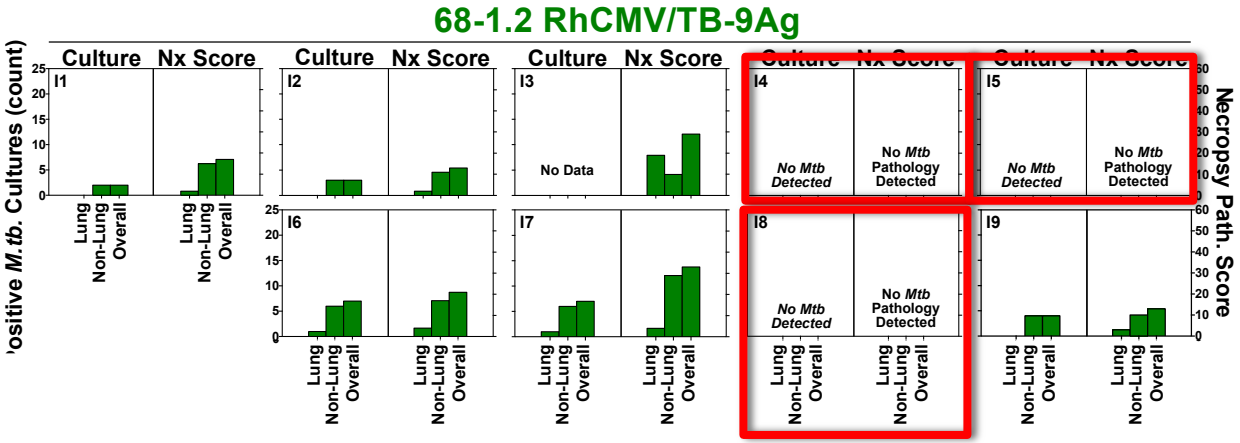
Both conventional and unconventionally targeted CD8+ T cells protect against TB





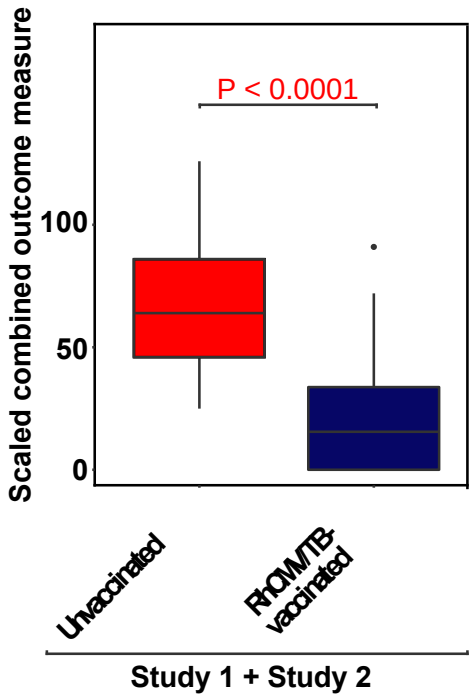
- Unvaccinated
- All RhCMV vaccinated
- 68-1 RhCMV/TB-9Ag
- 68-1.2 RhCMV/TB-9Ag
- 68-1 RhCMV/TB-6Ag

Disease at necropsy



Nature Medicine, 2018

Combined Outcome Analysis of RhCMV/TB Efficacy Studies #1 and #2



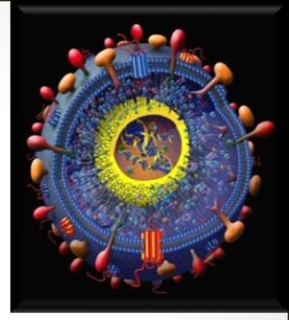
Using a scaled, combined (culture + path score) outcome measure, the extent of overall disease significantly reduced in RM vaccinated with RhCMV/TB alone compared to unvaccinated controls.

By Poisson modeling, the overall reduction in this combined outcome score over both studies was 68% (95% CI: 35.9-85.1%; P=0.0019).

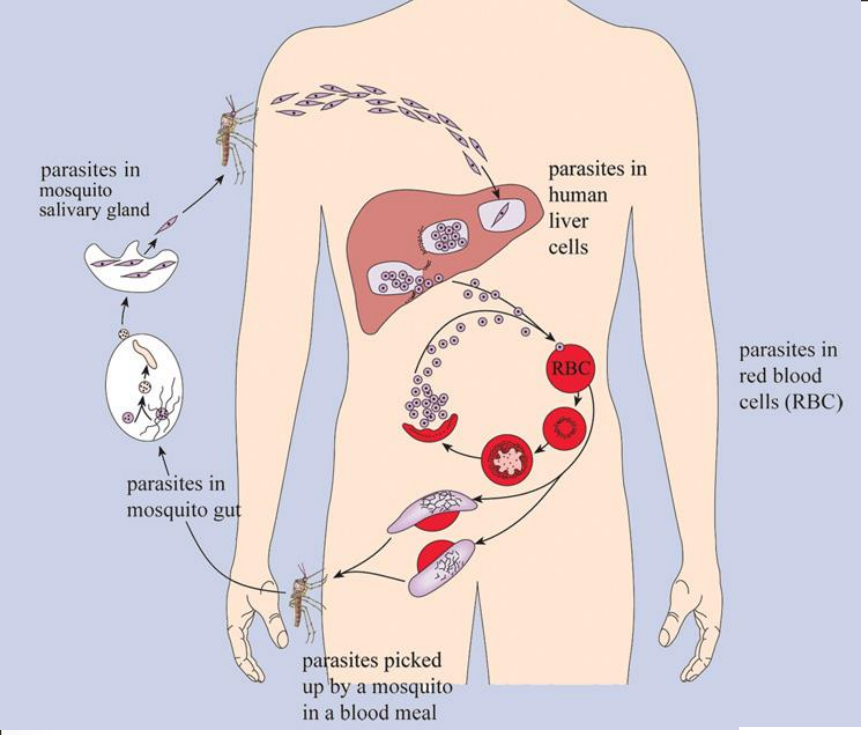
Note: no significant efficacy was observed with standard BCG vaccination

Currently comparing 68-1 and 68-1.2 6Ag in larger cohorts

Nature Medicine, 2018



Malaria



Plasmodium knowlesi

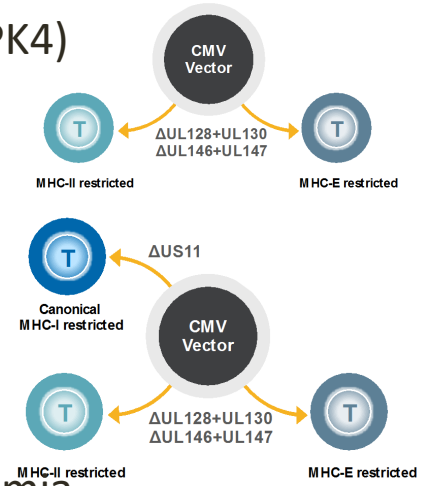
- wide-spread in SE Asia
- Zoonotic infection in humans
- Rhesus macaques not natural hosts (highly susceptible)

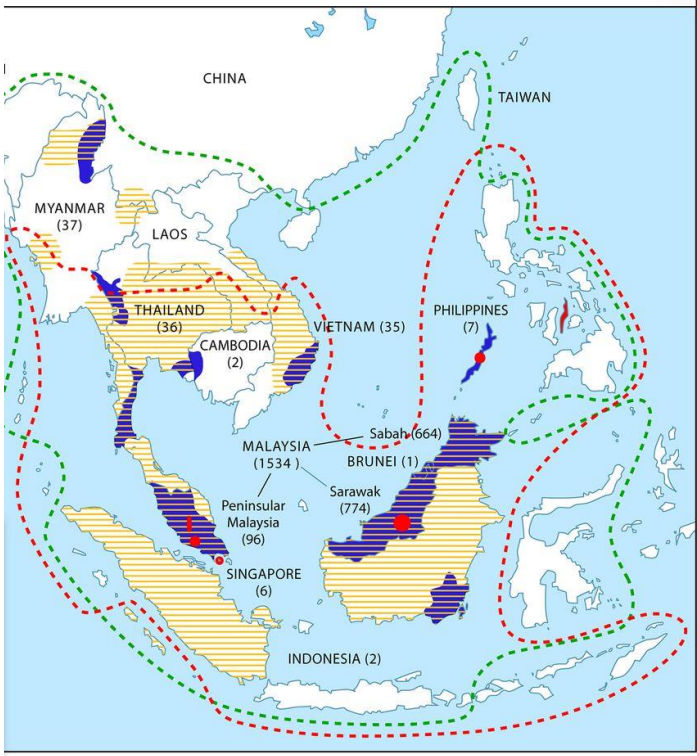
Vaccine: CSP/MSP1/TRAP/AMA1 (PK4)

- 68-1 RhCMV/PK4 (n=8)
- 68-1 RhCMVΔUS11PK4 (n=8)
- Control cohort (n=8)

Challenge

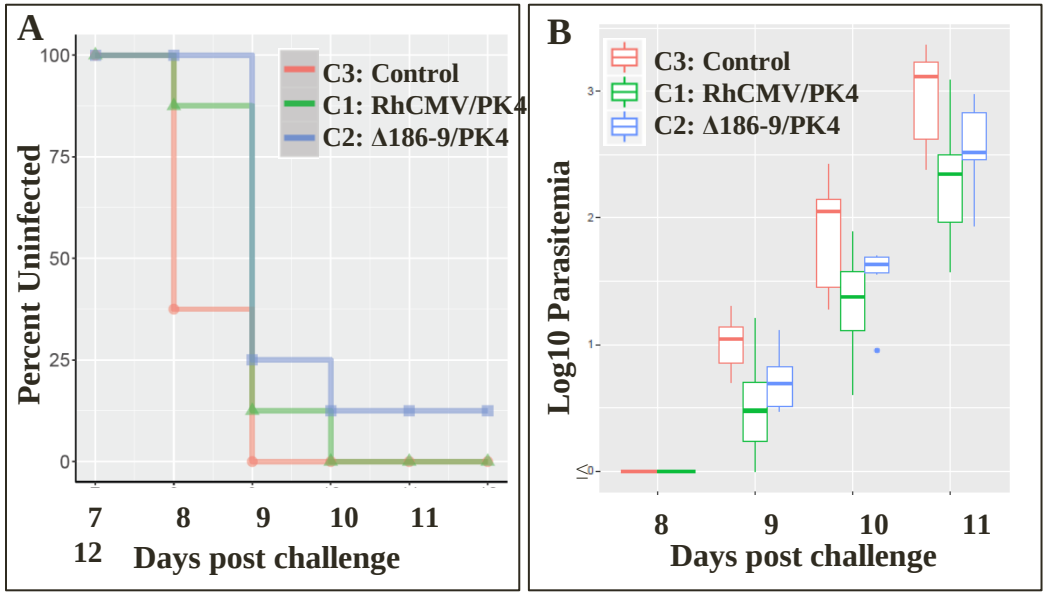
- 100 Sporozoites inoculated intravenously
- After day 6: monitor blood viremia by thin smear





unpublished

Challenge of RhCMV/PK4 immunized RM with *P. knowlesi* sporozoites



	Vaccine Efficacy (% reduction at day 8)			
Cohort	**VE**	**2.5%**	**97.5%**	**P value**
C3 vs. C1	**75%**	**49%**	**88%**	**0.0002**
C3 vs. C2	**80%**	**60%**	**90%**	**<0.0001**

Delay in appearance of blood stage parasites in both vaccine cohorts

~ 80% reduction of liver stage

No impact on blood stage

One sterile animal in ΔUS11 68-1 group

Currently testing additional antigen and vectors eliciting conventional CD8+ T cells

unpublished

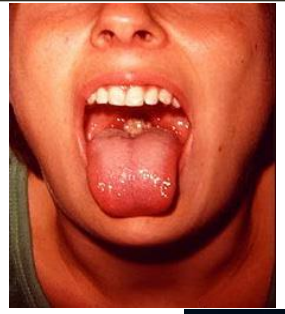
Not only is the answer to this question "yes", but the immunobiologic properties of CMV vectors turned out to be markedly different from any other known vector or non-vector vaccine modality . . .

In nonhuman primates, vectors based on rhesus cytomegalovirus:

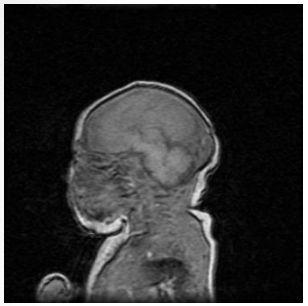
1. Elicit and indefinitely maintain high frequency, "effector memory" T cell responses* in mucosal sites, lymphoid tissues and parenchymal organs
2. Efficiently re-infect & persist despite robust anti-CMV immunity
3. Manifest uniquely programmable CD8+ T cell immunogenicity with expanded breadth and conventional and/or novel epitope targeting.
4. Provide protection against SIV, TB and Malaria
5. Maintain immunogenicity even with profound attenuation (spread-deficiency and tropism restriction)

*Little to no antibodies

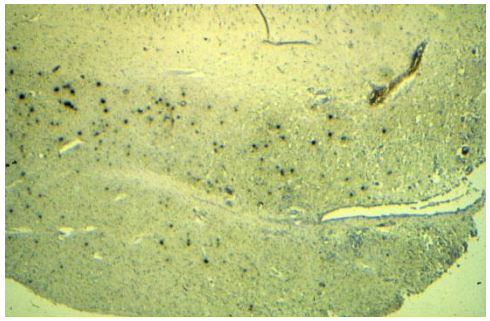
Since HCMV can be a pathogen, vector safety is a top priority issue . . .



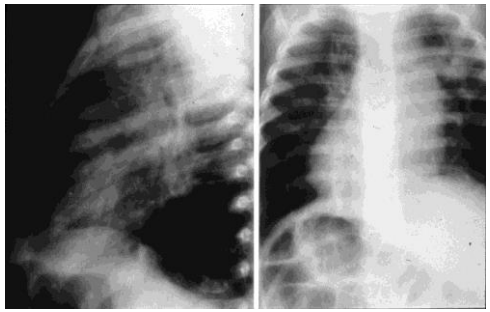
mononucleosis



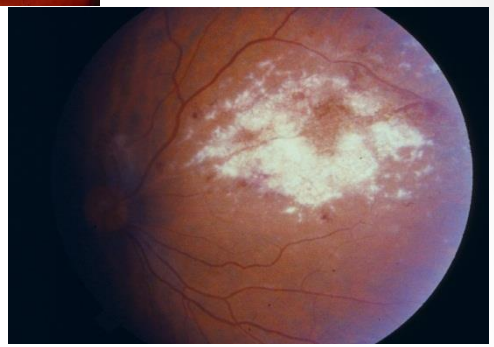
Congenital disease



encephalitis



pneumonitis



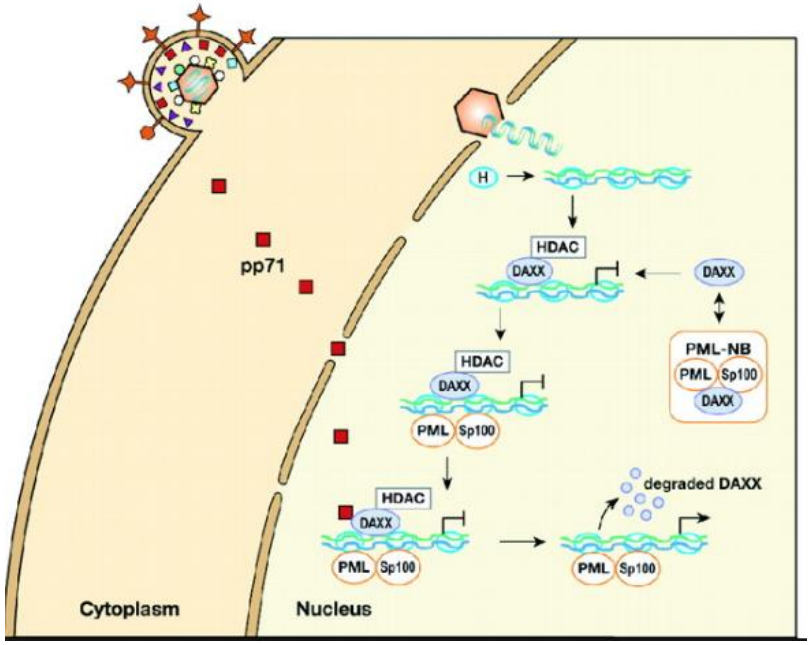
retinitis

Thus, for clinical translation, CMV vectors must be be engineered to be have:

- **greatly reduced or no ability to cause disease,**
- **greatly reduced or no ability for person-to-person transmission,**
- **stability *in vitro* and *in vivo*, and**
- **the ability to be GMP manufactured at large scale,**

While retaining the ability to super-infect, persist, and elicit/maintain all the immune responses needed for efficacy . . .

Employing intrinsic and innate immunity to attenuate CMV



Kalejta R F Microbiol. Mol. Biol. Rev. 2008;72:249-265

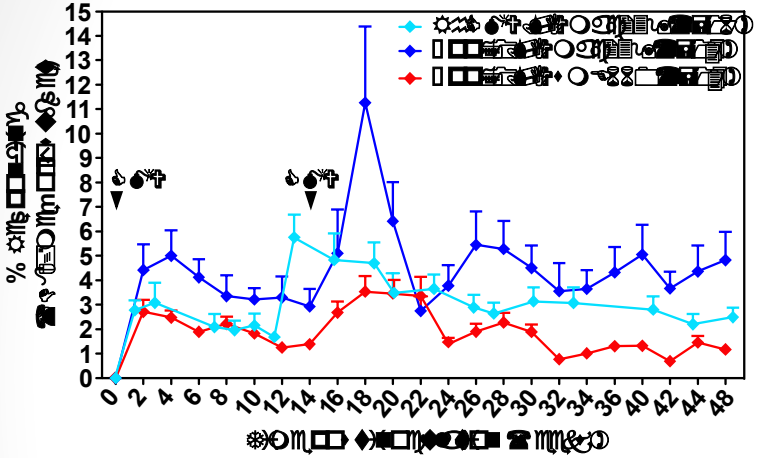
The CMV tegument protein pp71 counters DAXX, a host intrinsic immune protein that blocks the viral gene expression required for lytic infection.
pp71 has also been shown to counteract the innate immune signaling molecule STING

RhCMV lacking pp71 is highly attenuated in vivo:

- **Dissemination**: pp71-deleted RhCMV is limited to site of inoculation, very little dissemination
- **Shedding:** pp71-deleted RhCMV cannot be detected in urine by co-culture at any time post-infection
- **Transmission:** pp71-deleted RhCMV is not transmitted upon leukocyte transfusion, pp71-deleted RhCMV is not transmitted via breast milk from mother to infant
- **Fetal inoculation**: reduced spreading and fetal loss compared to wildtype RhCMV upon direct inoculation of high doses (10e6) of pp71-deleted RhCMV

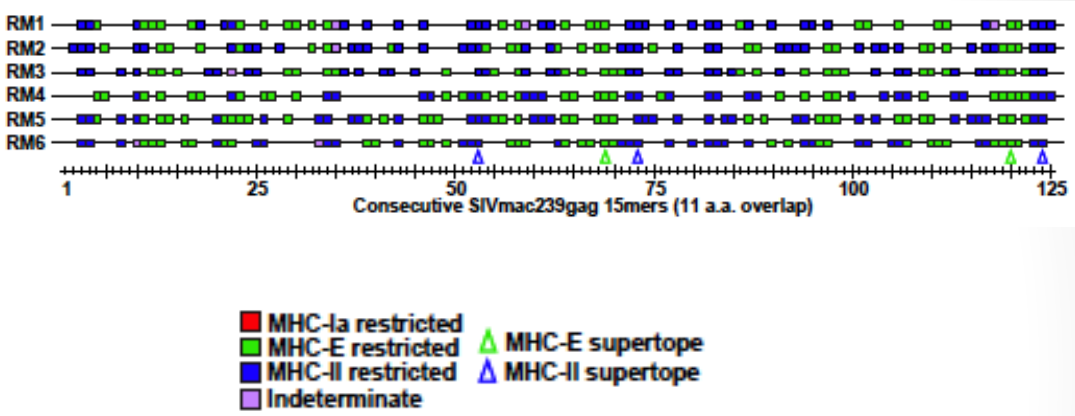
Yet, Δpp71 68-1 RhCMV/SIV vectors are similarly immunogenic and protective as WT 68-1 vectors, including against heterologous challenge

RhCMV/SIV vector-elicited CD8+ T cell responses to SIVmac239 sequence peptides (total)



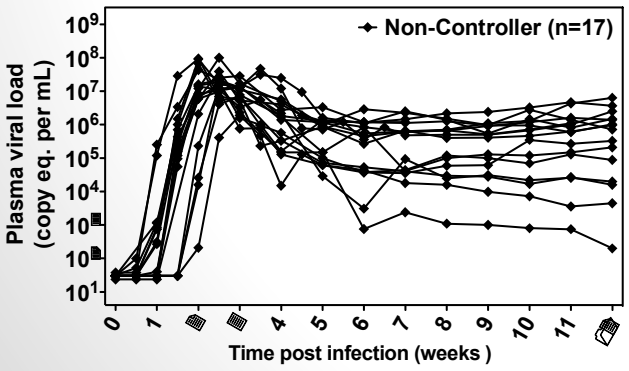
[RhCMV inserts: Gag, Pol, Rev/Nef/Tat, Env]

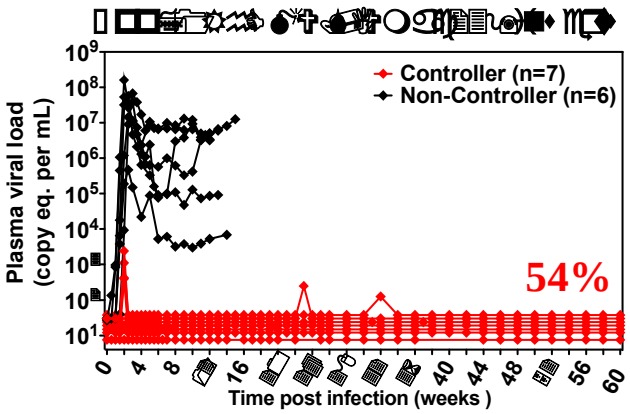
Epitope Targeting of pp71-deleted 68-1 RhCMV

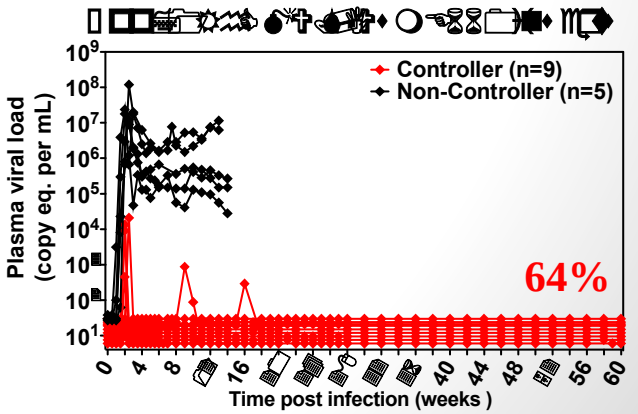


Outcome of SIVmac239 challenge:

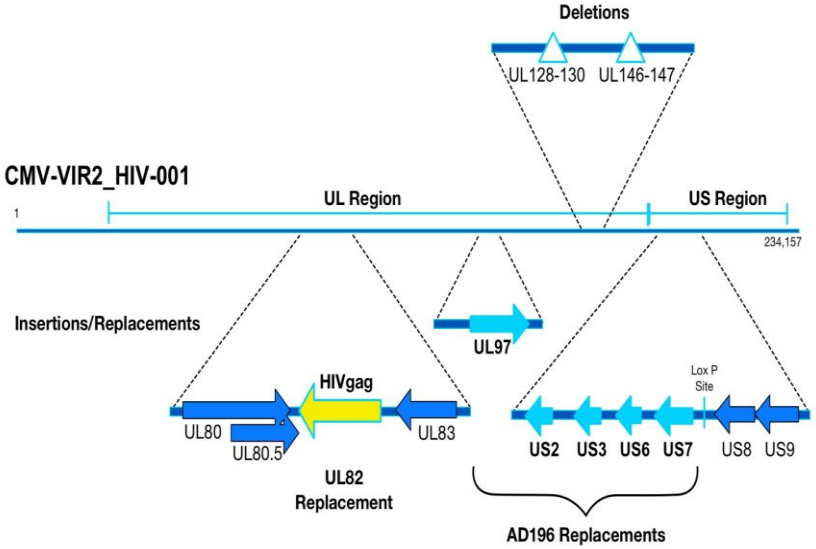
Unvaccinated controls







Based on RhCMV results we now generated a pp71-deleted "68-1" like HCMV/HIV vector for clinical testing



Vector = Vaccine Prototype designed to determine

a) **Safety**
b) **Reinfection capability**
c) **Immunogenicity (CMV, HIV, T cell phenotype)**
d) **Immune Programming**

<u>Characteristics of pp71-deleted HCMV</u>:

-highly attenuated in vitro (4log at low MOI in fibroblasts)
-reduced ability to infect non-fibroblast cells (due to UL128-130 deletion)
-maintains ability to establish latency in humanized mouse model
-reduced ability to reactivate in humanized mouse model
-immunogenic in monkeys >10e5 FFU

This vector is now in GMP manufacturing with clinical testing planned for 2019

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Global HIV Vaccine Enterprise

VIR

OHSU has licensed CMV technology, of which Dr. Frueh is an inventor, to VIR Biotechnology, a company in which both OHSU and Dr. Frueh have significant financial interest. Potential individual and institutional conflicts of interest have been reviewed and are actively managed by OSHU.

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