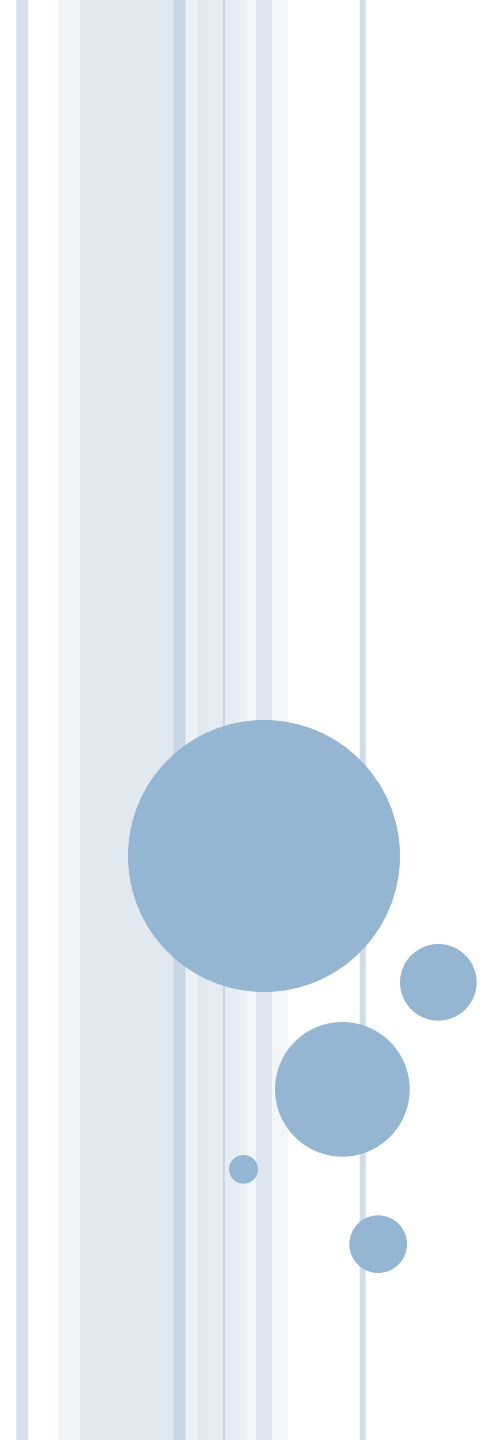


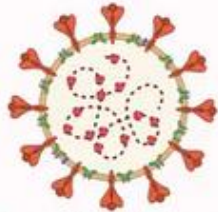


DRUG AND VACCINE TREATMENTS

Unit 7
Paul Thomas
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Vaccine and Infectious Disease Division
Fred Hutchinson Cancer Center

a. Inactivated vaccines

Inactivated vaccines contain SARS-CoV-2 viruses that are chemically inactivated



b. Recombinant proteins vaccines

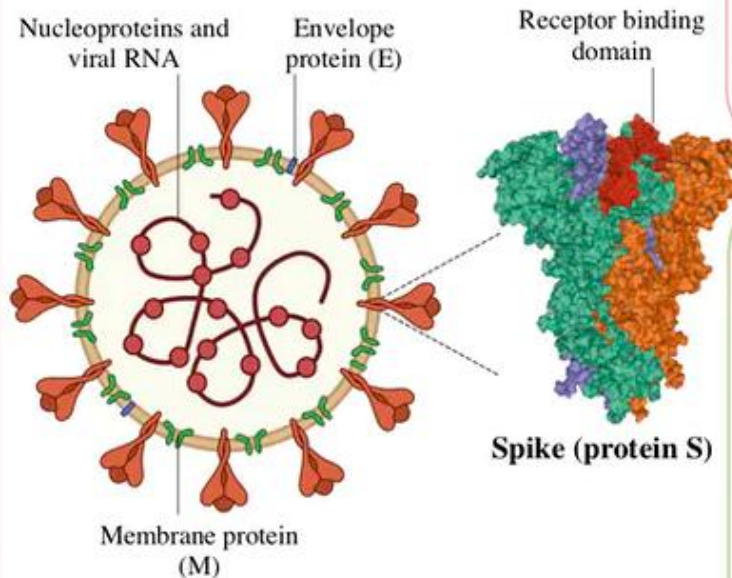
Vaccines composed of recombinant spikes
Vaccines composed of receptor binding domain



NovaVax



Virus-like particles are devoid of genetic material but display spikes, M and E proteins on their surface

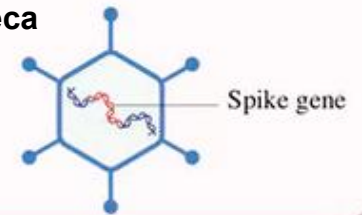


SARS-CoV-2

c. Viral vector vaccines

Viral vector vaccines contain another virus modified to express S protein

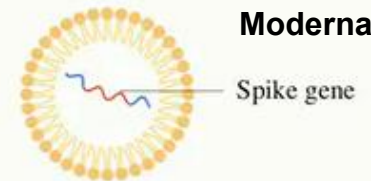
**JNJ
AstraZeneca**



d. RNA vaccines

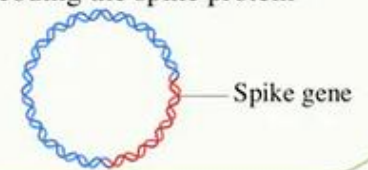
RNA vaccines consist of RNA packed in lipid nanoparticles

**Pfizer/BioNTech
Moderna**



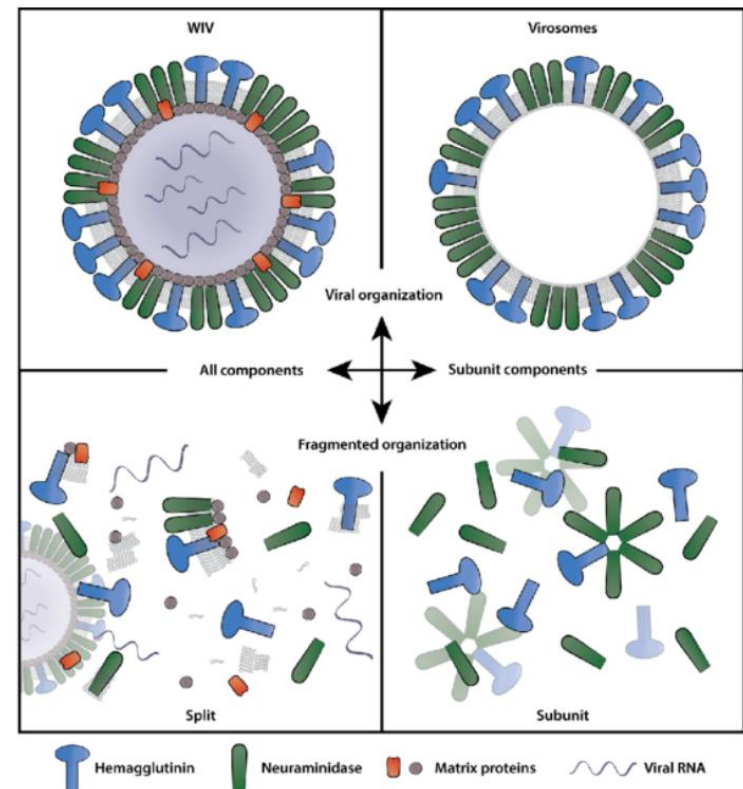
e. DNA vaccines

DNA vaccines contain a circular DNA encoding the spike protein



COMPARISON: ANNUAL “FLU SHOT” QIV

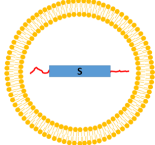
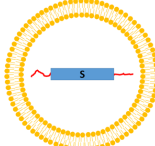
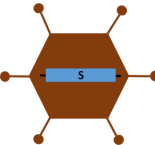
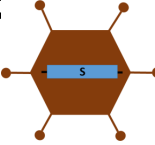
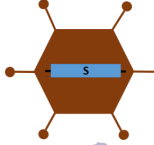
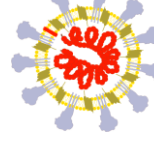
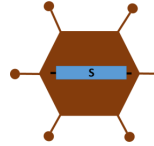
- The annual flu shot is generated by inactivating a whole, attenuated virus, fragmenting it with detergent, and reforming virosomes missing the viral RNA and most viral proteins
- There is **NO ADJUVANT**



Data from Phase I/II trials

Company (reference)	Vaccine (type)	Dose range (route)	Neut. titre after prime	Neut. titre after boost	T cell response	Trial registration number
Sinovac ³⁵	CoronaVac (inactivated SARS-CoV-2 + aluminium hydroxide)	3–6 µg (i.m.) 2x	ND	1:30–1:60 range ^a	ND	NCT04352608
Sinopharm	Inactivated whole virus COVID-19 vaccine (inactivated SARS-CoV-2 + aluminium hydroxide)	2.5, 5 or 10 µg (i.m.) 3x (0/28/56 or 0/28) 5 µg (i.m.) 2x (0/14 or 0/21)	Not reported in detail	1:316 (2.5 µg, 0/28/58) ^c 1:206 (5 µg, 0/28/58) ^c 1:297 (10 µg, 0/28/58) ^c 1:121 (5 µg, 0/14) ^c 1:247 (5 µg, 0/21) ^c	ND	ChiCTR2000031809
CanSino ⁴⁶	Ad5 nCoV (non-replicating AdV5 expressing spike protein)	5 x 10 ¹⁰ , 10 ¹¹ VP (i.m.)	1:18.3–1:19.5 range ^b	—	Yes	NCT04341389
AstraZeneca ⁴⁷	ChAdOx1nCoV-19 (non-replicating chimpanzee AdV expressing spike protein)	5 x 10 ¹⁰ VP 1 x or 2' (i.m.)	Median 1:218 ^c Median 1:51 ^d Median 1:4–1:16 ^e	Median 1:136 ^d Median 1:29 ^d	Yes	NCT04324606
Moderna ⁵⁹	mRNA-1273 (mRNA)	2x 25, 100 , 250 µg (i.m.)	Low	1:112.3 (25 µg) ^f 1:343.8 (100 µg) ^f 1:332.2 (250 µg) ^f 1:339.7 (25 µg) ^g 1:654.3 (100 µg) ^g	Good CD4 ⁺ and low CD8 ⁺ response	NCT04283461
Pfizer ⁶⁰	BNT162b1 (mRNA)	2x 10, 30, 100 µg (i.m.)	Low	1:180 (10 µg) ^h 1:437 (30 µg) ^h	ND	NCT04368728
Pfizer ⁸⁴	BNT162b1 (mRNA) and BNT162b2 (mRNA)	2x 10, 20, 30 µg	Low	Day 28 ^h BNT162b1 (18–55 years): 1:168 (10 µg) 1:267 (30 µg) BNT162b1 (65–85 years): 1:37 (10 µg) 1:179 (20 µg) 1:101 (30 µg) BNT162b2 (18–55 years): 1:157 (10 µg) 1:363 (20 µg) 1:361 (30 µg) BNT162b2 (65–85 years): 1:84 (20 µg) 1:147 (30 µg)	ND	NCT04368728
Novavax ⁹⁰	NVX CoV2373 (Matrix-M) Spike protein 'rosettes'	2 x 2.5–25 µg (i.m. ± Matrix-M) 1x 25 µg (i.m. + Matrix-M)	1:128 (25 µg + Matrix-M) ⁱ	1:3,906 (5 µg + Matrix-M) ^j 1:3,305 (25 µg + Matrix-M) ^j 1:41 (25 µg unadjuvanted) ^j	CD4 ⁺	NCT04368988

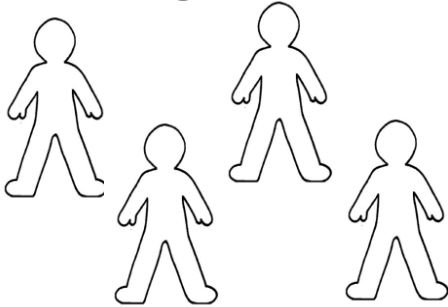
VACCINE EFFICACY IN PHASE III

- Moderna (94)%  
- Pfizer (95%)  
- AstraZeneca (62-90-70)  
- Janssen (72%)  
- Novavax (89-96%)  
- Gamaleya (91.6%)  
- Sinovac/Sinopharm (3x) (50-90%)  
- Cansino 

For most of these vaccines two injections are required.

HOW DOES A PHASE III STUDY WORK?

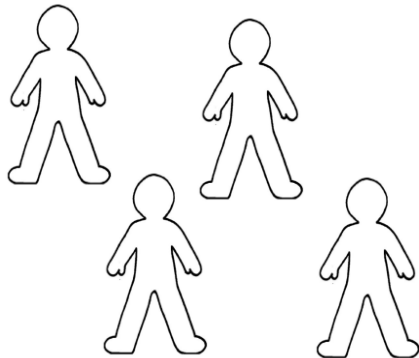
Vaccine group



Conducted by independent medical centers (usually geographically distributed)

An independent committee watches the data

Analysis timepoints and success are pre-defined

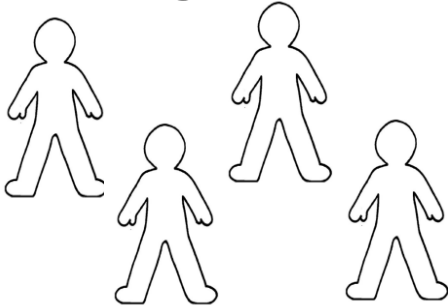


Placebo control group

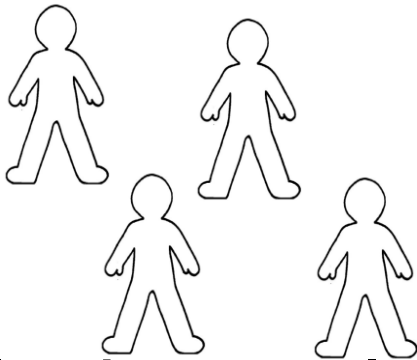
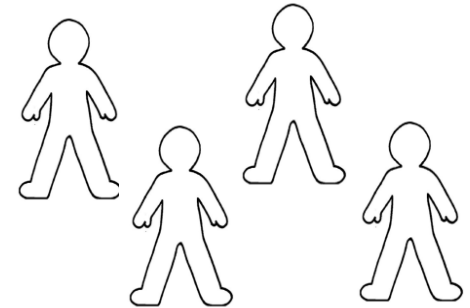
Special thanks to Florian Kramm

HOW DOES A PHASE III STUDY WORK?

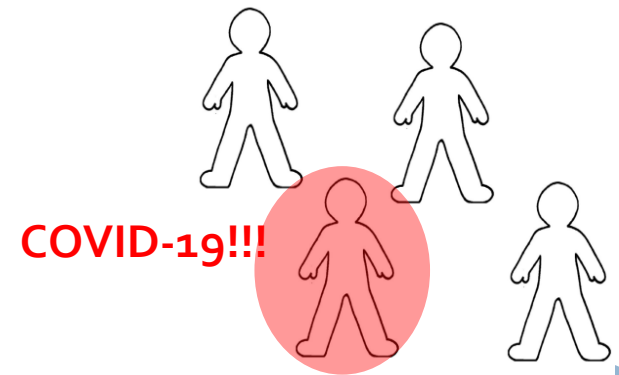
Vaccine group



Time

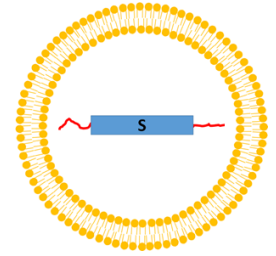


Placebo control group



Special thanks to Florian Kramm

WHAT DO THE PFIZER RESULTS MEAN?

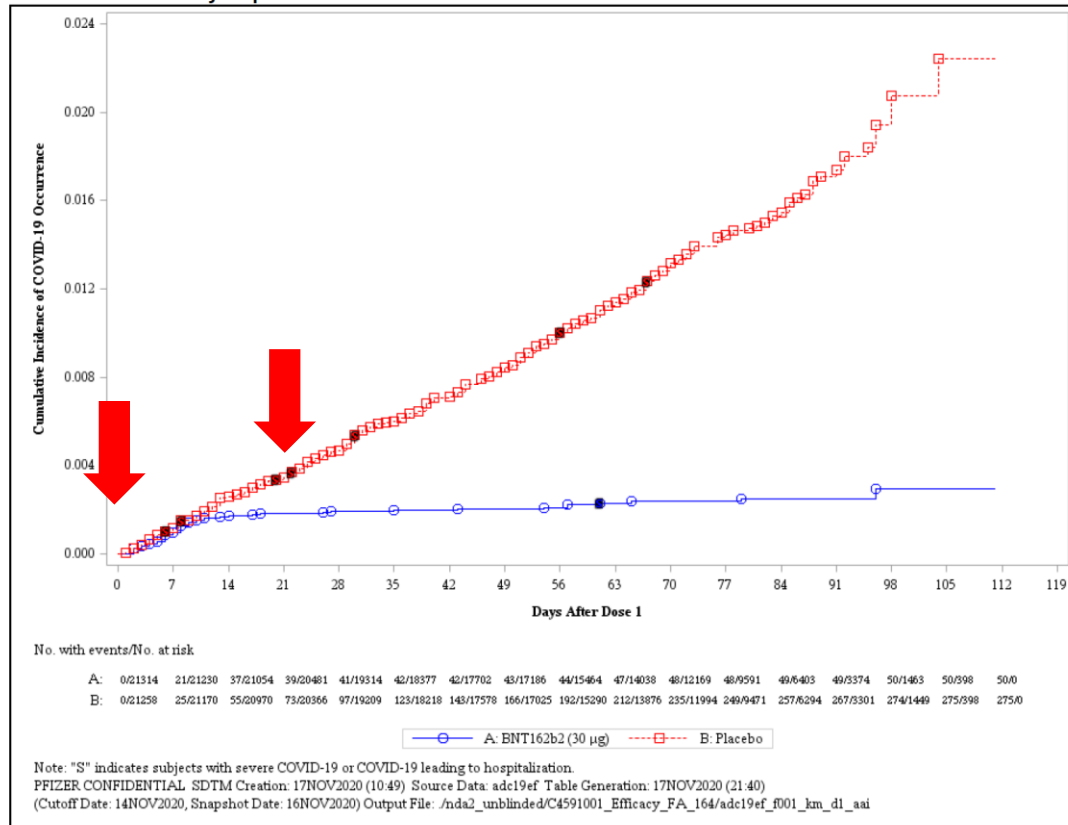


- 43,538 individuals are in the study
- 170 COVID-19 cases were recorded
 - 162 in the placebo group (9 severe)
 - 8 in the vaccine group (1 severe)
- 95% efficacy against symptomatic disease (one symptom plus PCR+, they start measuring this 7 days post dose 2)
- 94% efficacy in the 65-85 year old group
- No significant safety concerns

- The vaccine received different degrees of approval in Bahrain, the UK, Mexico, Canada, Saudi Arabia, the EU, the US etc.

Moderna data look almost identical

Figure 2. Cumulative Incidence Curves for the First COVID-19 Occurrence After Dose 1, Dose 1 All-Available Efficacy Population



<https://www.fda.gov/media/144245/download> accessed 8Dec20

Special thanks to Florian Kramm

RNA vaccines are a relatively new development

RNA vaccine trials in humans

(not including a large number of cancer vaccines and therapeutic approaches based on mRNA)

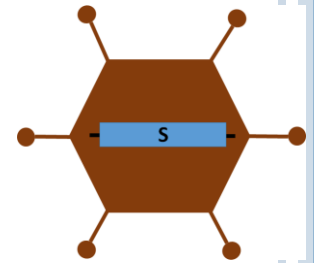
Target	Started in	Individuals enrolled ²	Company	Status	Phase	Registration number
CMV	2017	181	Moderna	Fully enrolled	Phase 1	NCT03382405
hMPV/PIV3	2019	114	Moderna	Recruiting	Phase 1	NCT04144348
Zika	2019	120	Moderna	Fully enrolled	Phase 1	NCT04064905
Influenza	2017	156	Moderna	Fully enrolled	Phase 1	NCT03345043
Rabies	2018	53	Curevac	Fully enrolled	Phase 1	NCT03713086
Rabies	2013	101	Curevac	Completed	Phase 1	NCT02241135
Rabies	2014	72	Curevac	Completed	Phase 1	NCT02238756
CMV	2020	452	Moderna	Recruiting	Phase 2	NCT04232280
Chikungunya ¹	2019	39	Moderna	Fully enrolled	Phase 1	NCT03829384

¹Passive immunity based on *in vivo* mAb expression

²Includes individuals who received placebo, some trials are still recruiting

Special thanks to Florian Kramm

WHAT DO THE J&J RESULTS MEAN?



- One dose!
- 43,783 individuals are in the study
- USA, South Africa and Latin America
- US efficacy 72% against moderate to severe COVID-19 (2 symptoms plus PCR+ was counted as moderate)
- 85% efficacy across all studies against severe disease
- 100% protection against hospitalization and death
- No significant safety concerns
- Some indication of reduction of asymptomatic infections

ARE VECTORED VACCINES A RELATIVELY NEW DEVELOPMENT?

- Ad26-based Ebola vaccine licensed in the EU
- Ad4 and Ad7 vaccines in use in the US military since 1971



Reactogenicity

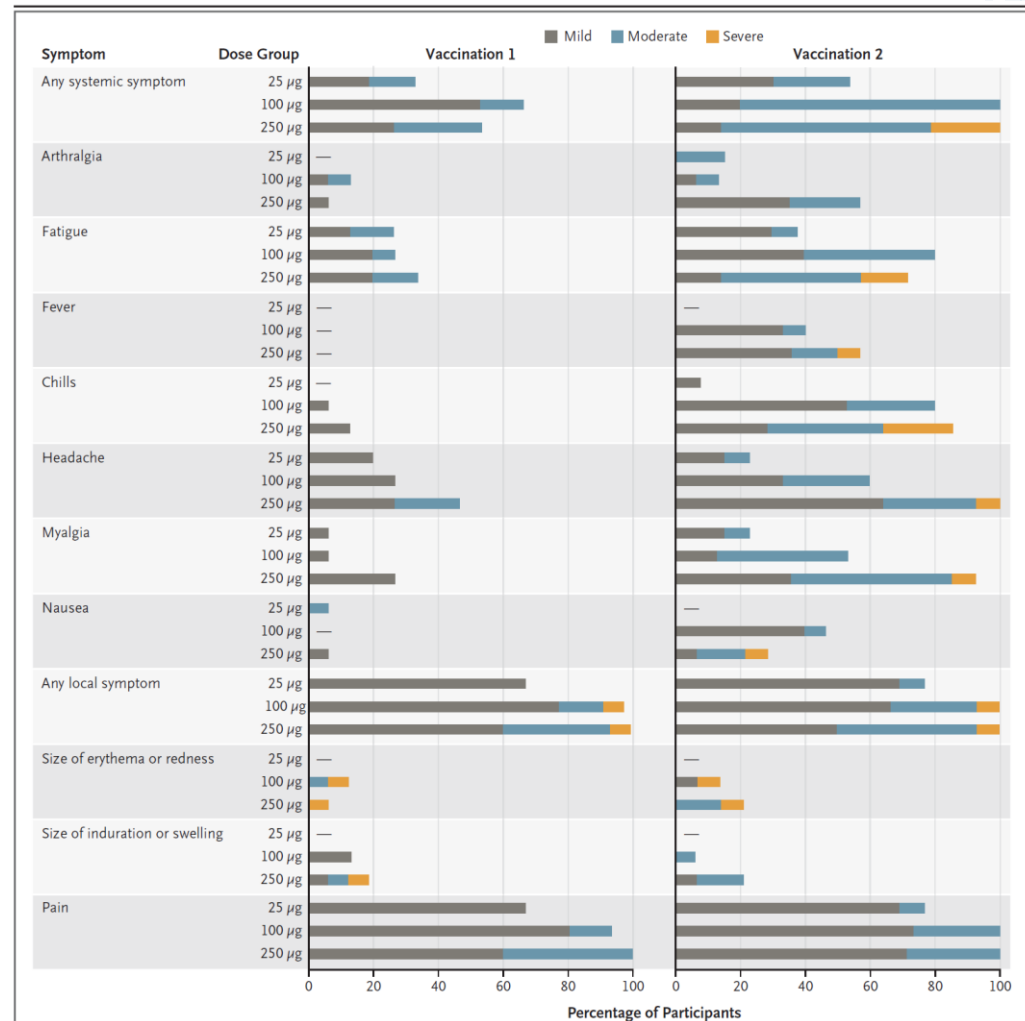
- Injection site pain
- Headache
- Fatigue
- Elevated temperature
- Myalgia
- Mild flu-like symptoms

→ unpleasant, but not dangerous

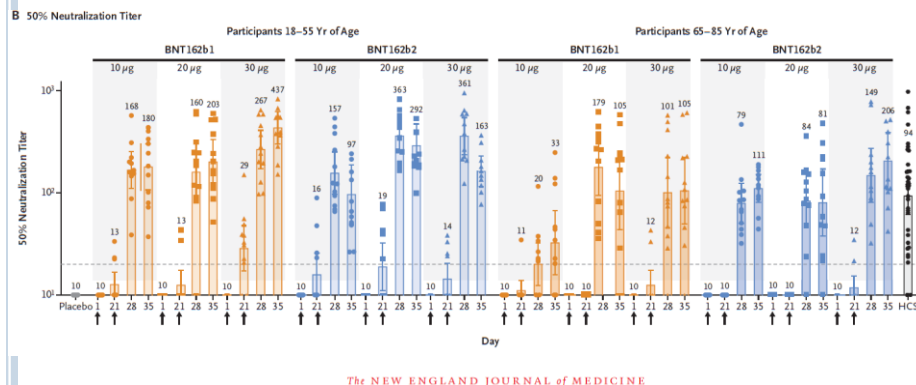
AdV=mRNA>recombinant protein>inactivated vaccine

Strength of adjuvant!

Moderna/VRC mRNA 1273 via LNPs



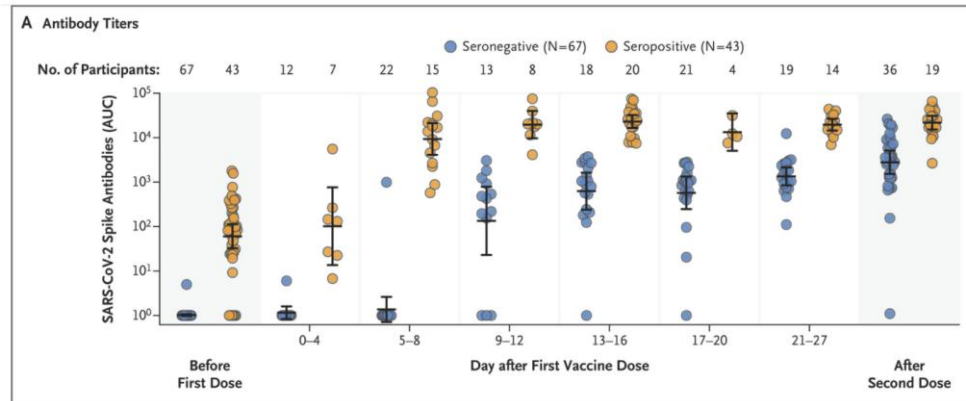
VACCINES WORK IN OLDER INDIVIDUALS AND BOOST MEMORY IN INFECTED INDIVIDUALS



ORIGINAL ARTICLE

Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates

- Vaccines work faster in younger individuals and with lower doses
- With recommended dose, older individuals still generate high levels of protective immunity



CORRESPONDENCE

Antibody Responses in Seropositive Persons after a Single Dose of SARS-CoV-2 mRNA Vaccine

- Post-infection, a single dose of the Pfizer/BioNTech vaccine was equivalent to two doses of the vaccine in naïve individuals
- Still a significant boost!

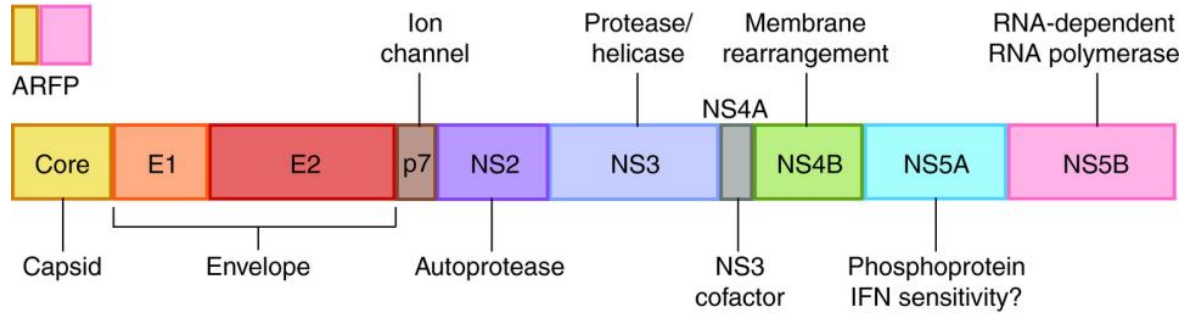
HEPATITIS C VIRUS

- Enveloped, positive strand RNA virus, *Flaviviridae*
- Isolated in 1989, treatments first emerged in early 1990s
- ~120 million-200 million infections worldwide, number one indication for liver transplant in the U.S.
- 10^{12} viral particles produced/day, $\frac{1}{2}$ life 3 hours in circulation
- Six major genotypes, 3 dominate in the U.S. (1, 2, 3)
 - 30-50% genetic variation among genotypes
 - 1-5% variation among viruses within a single patient
- Replicates via negative-stranded RNA in membranous web in cytoplasm

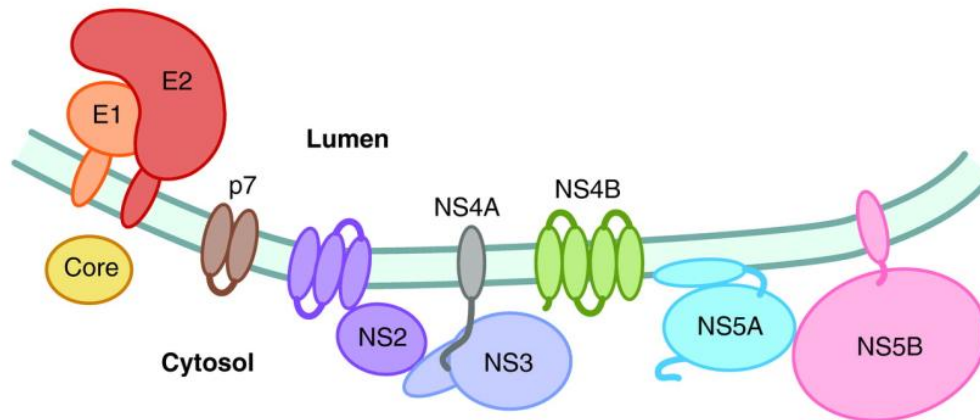


HCV STRUCTURE

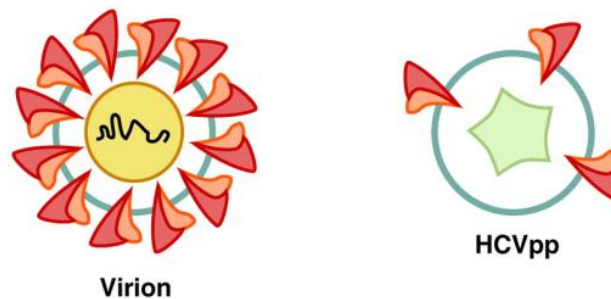
b



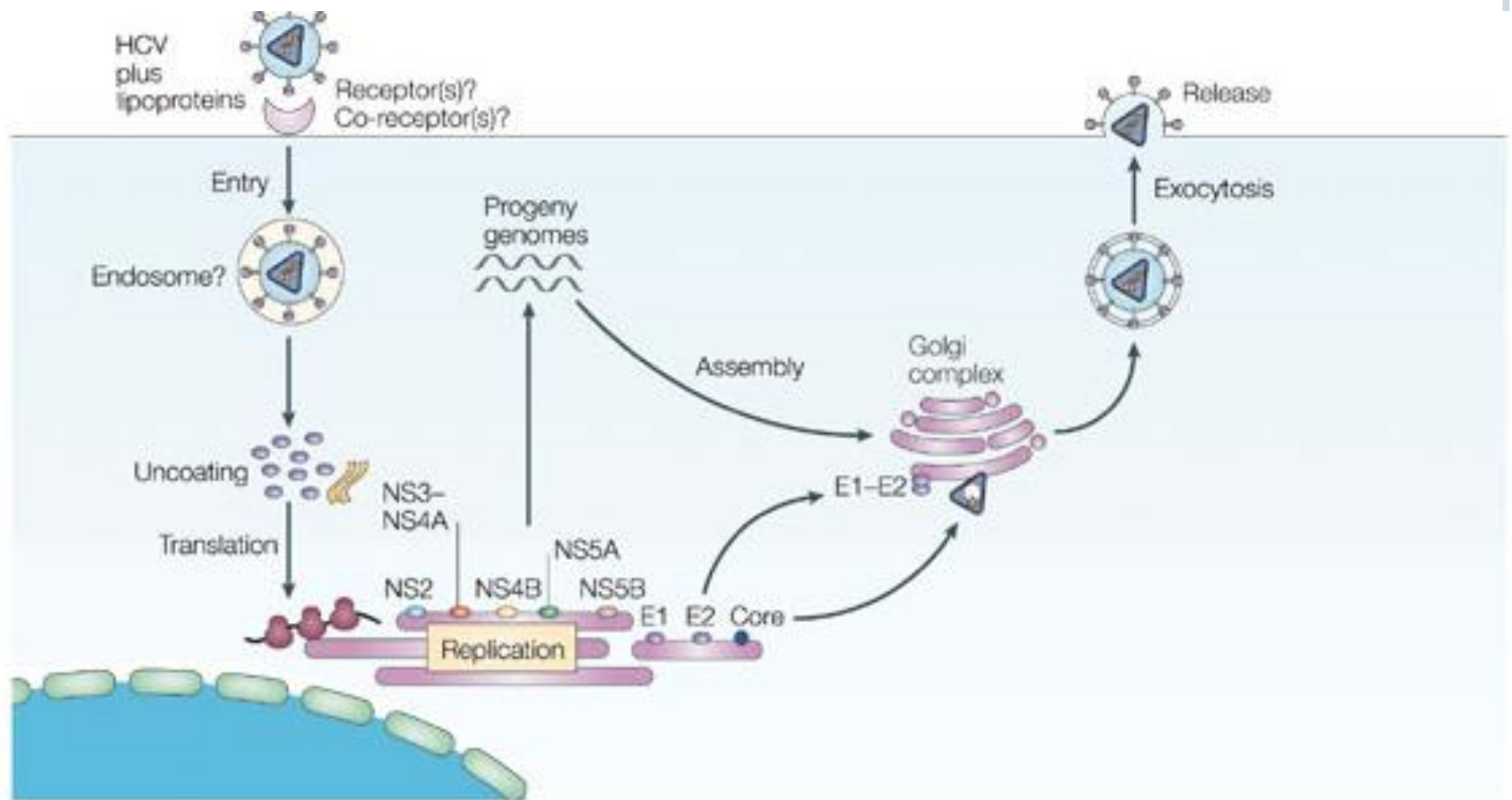
c



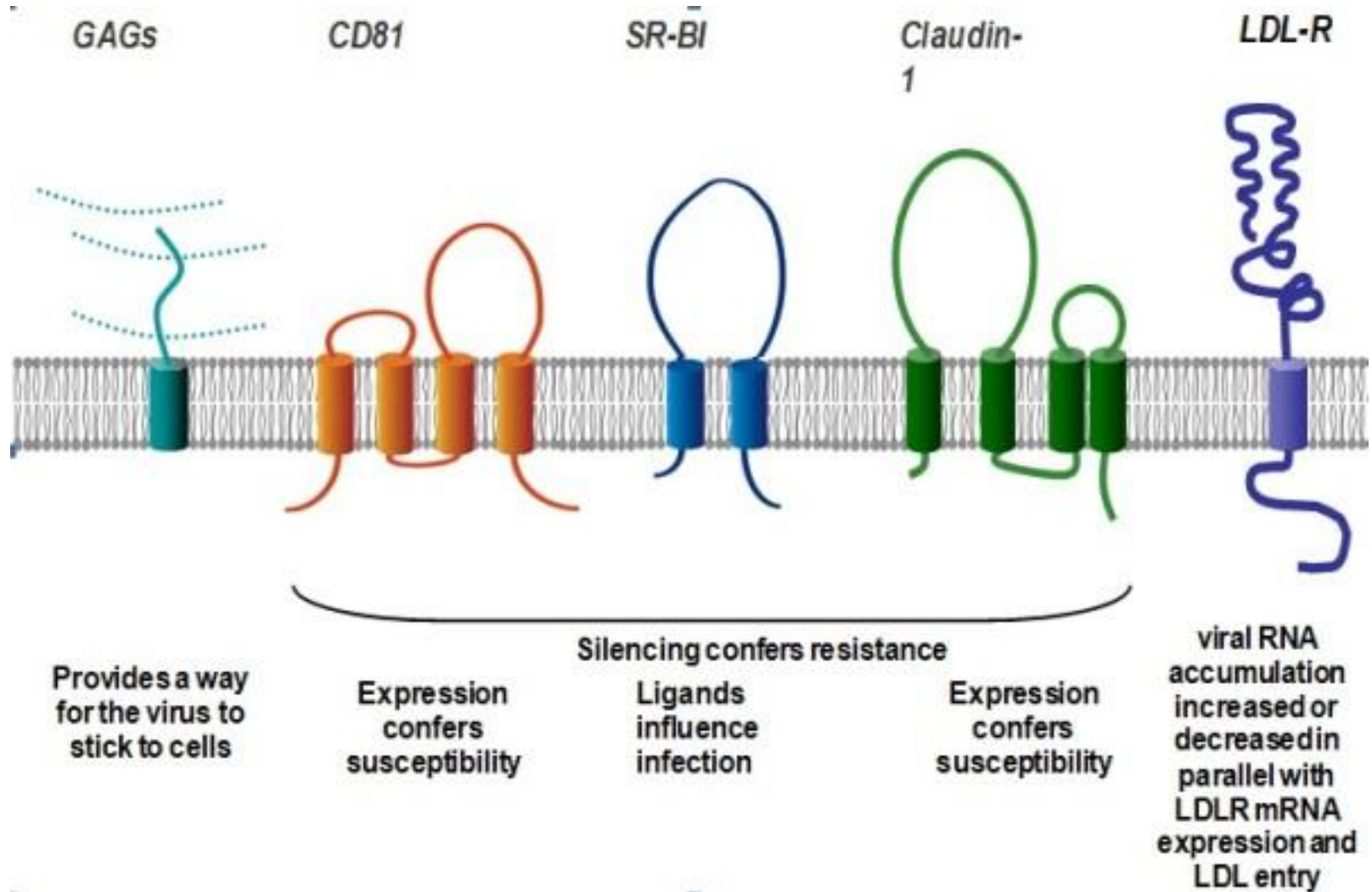
d



HCV LIFE CYCLE



RECEPTORS FOR VIRAL ENTRY

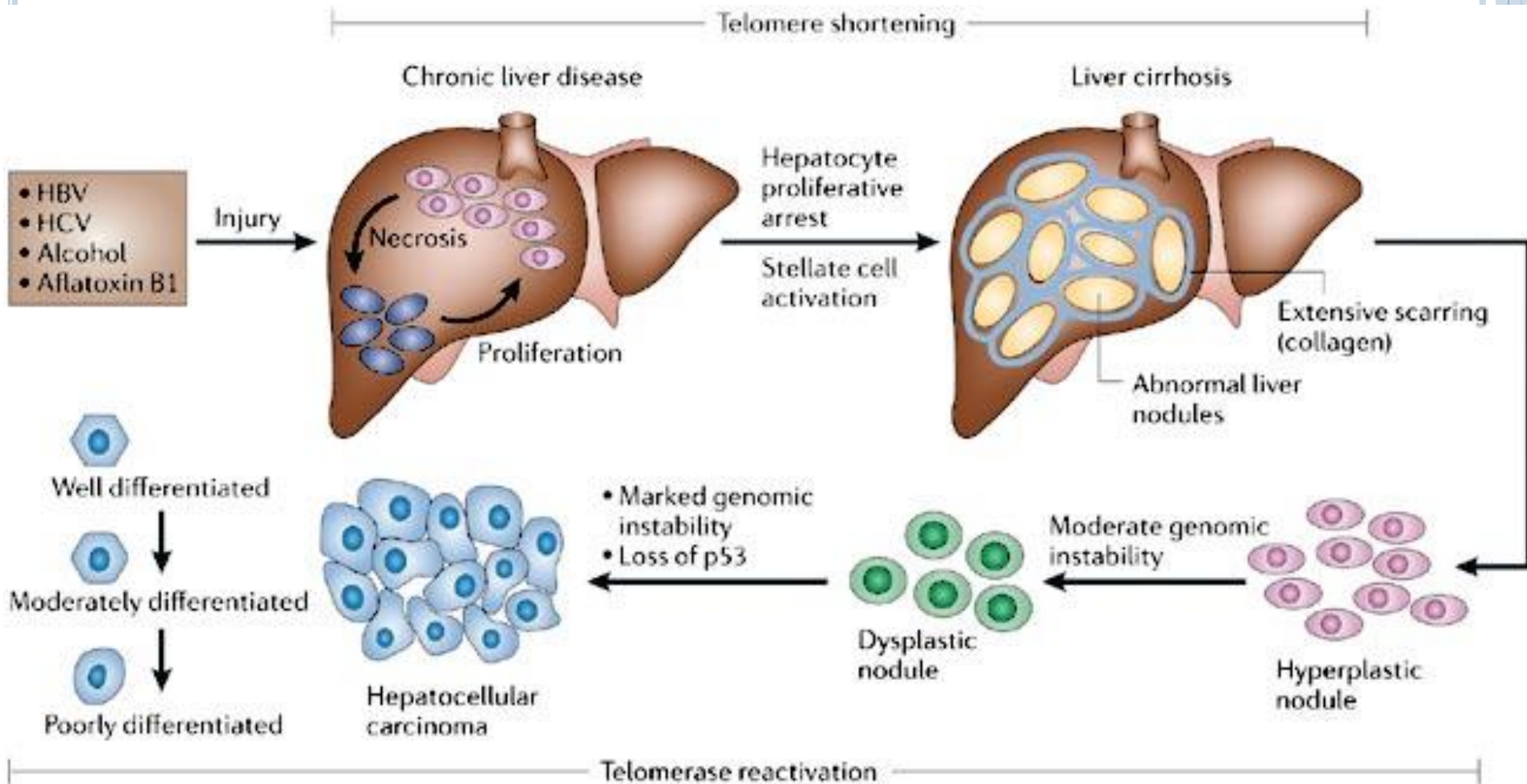


HCV receptors for cell entry.

Ashfaq *et al. Virology Journal* 2011 8:161 doi:10.1186/1743-422X-8-161

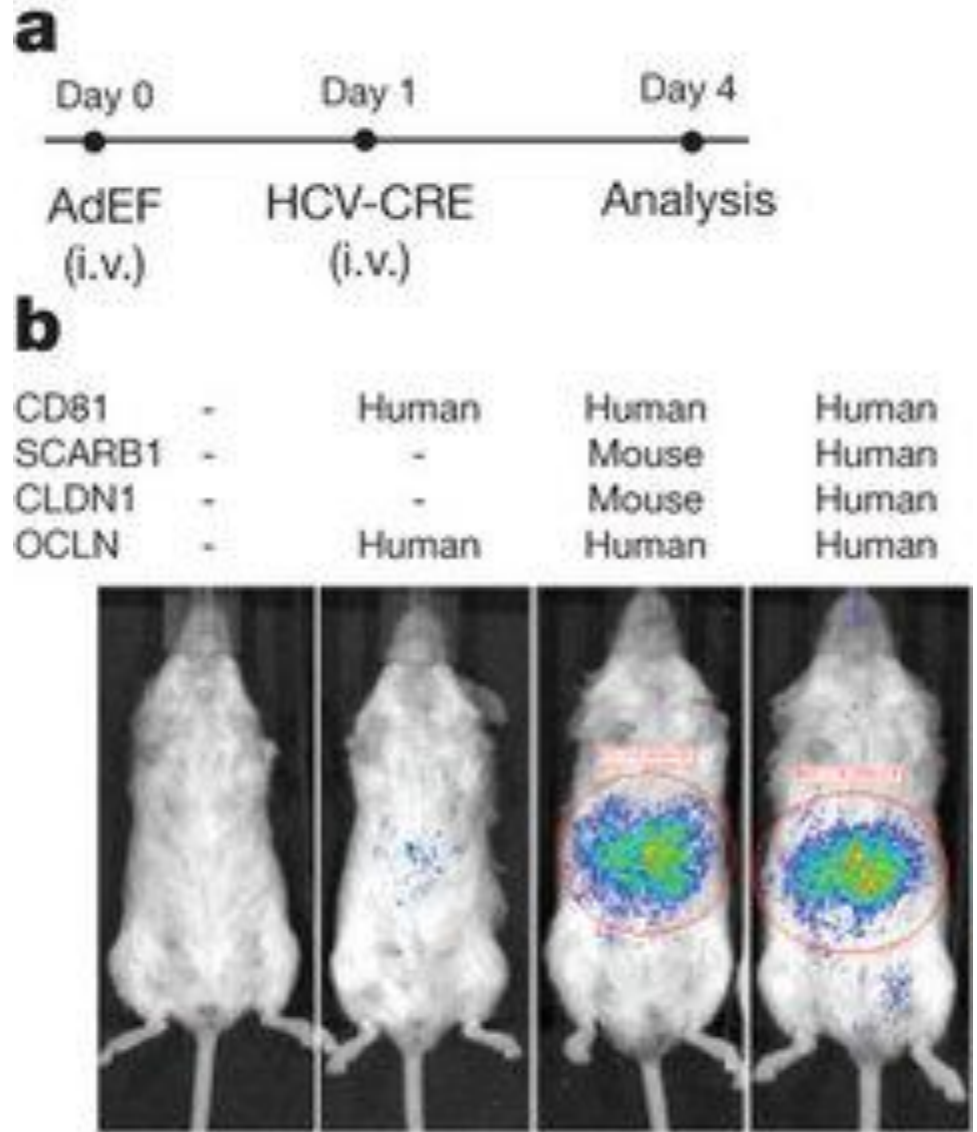
HCV LIFE CYCLE 2

- HCV-associated disease results from viral persistence leading to long term inflammation and cell turnover



MOUSE MODEL OF HCV REPLICATION

- Previous models relied human liver transplant into immunodeficient mice—limited usefulness
- Transgenic approach using four known entry factors—Occludin, CD81, SCARB and claudin 1



c

A genetically humanized mouse model for hepatitis C virus infection
Nature 474, 208–211 (09 June 2011)

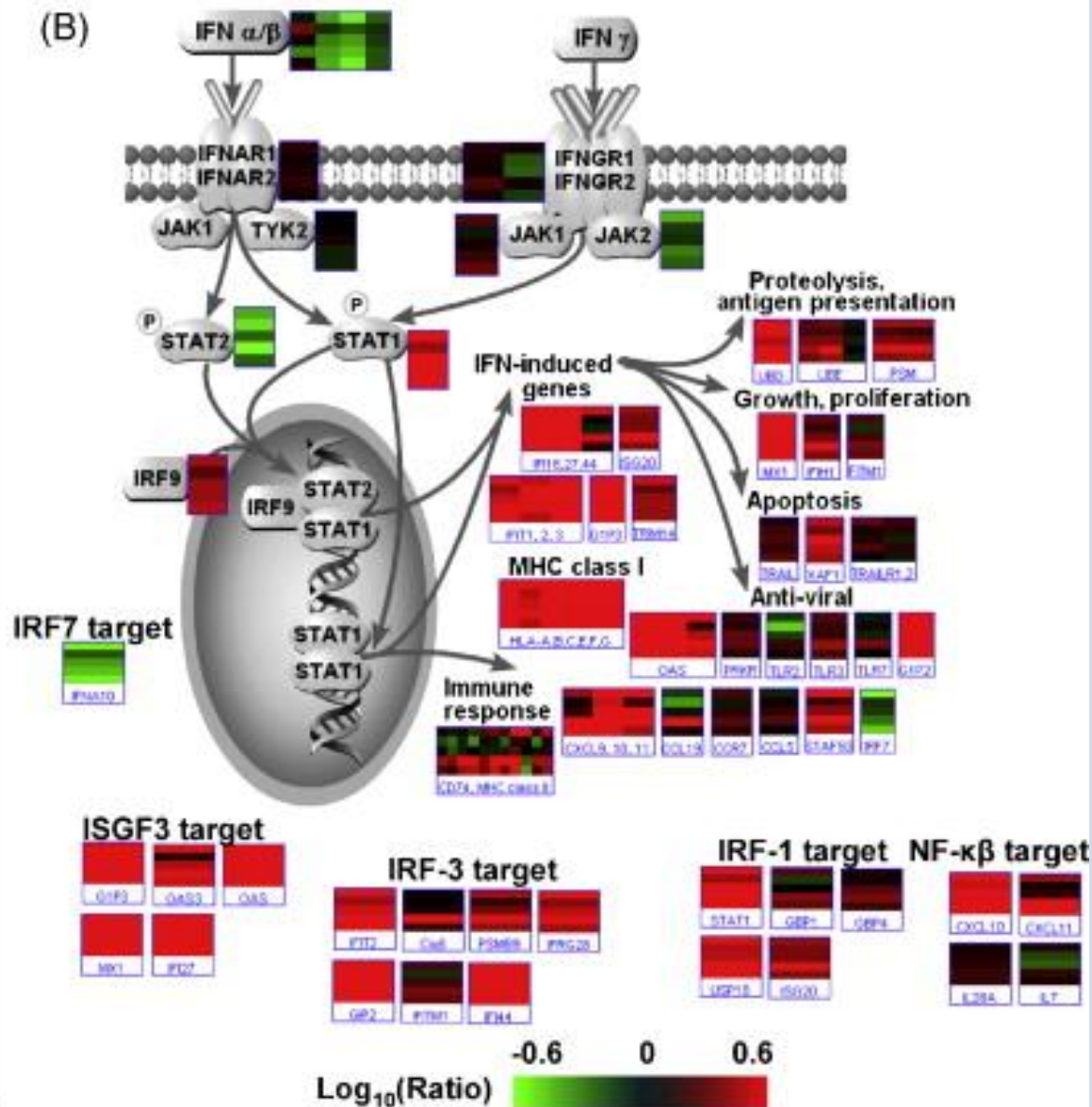
WHAT ARMS OF THE IMMUNE RESPONSE ARE USEFUL AGAINST HCV?

- Innate immunity
 - Antiviral effectors such as IFN that act on host cells, regulating key components of cell biology to limit viral growth and spread
- Antibody-mediated clearance
 - In principle, antibodies should be able to remove virus as it spreads from cell to cell
 - In practice, the correlation of antibody with HCV clearance and outcome is controversial or lacking
 - Patients with high levels of *neutralizing* antibodies nevertheless maintain chronic infection, indicating that neutralizing antibodies are not *sterilizing*
- Cell-mediated clearance
 - Infected cells can be killed before releasing progeny virions
 - Thought to be the primary means of long term control in HCV infection



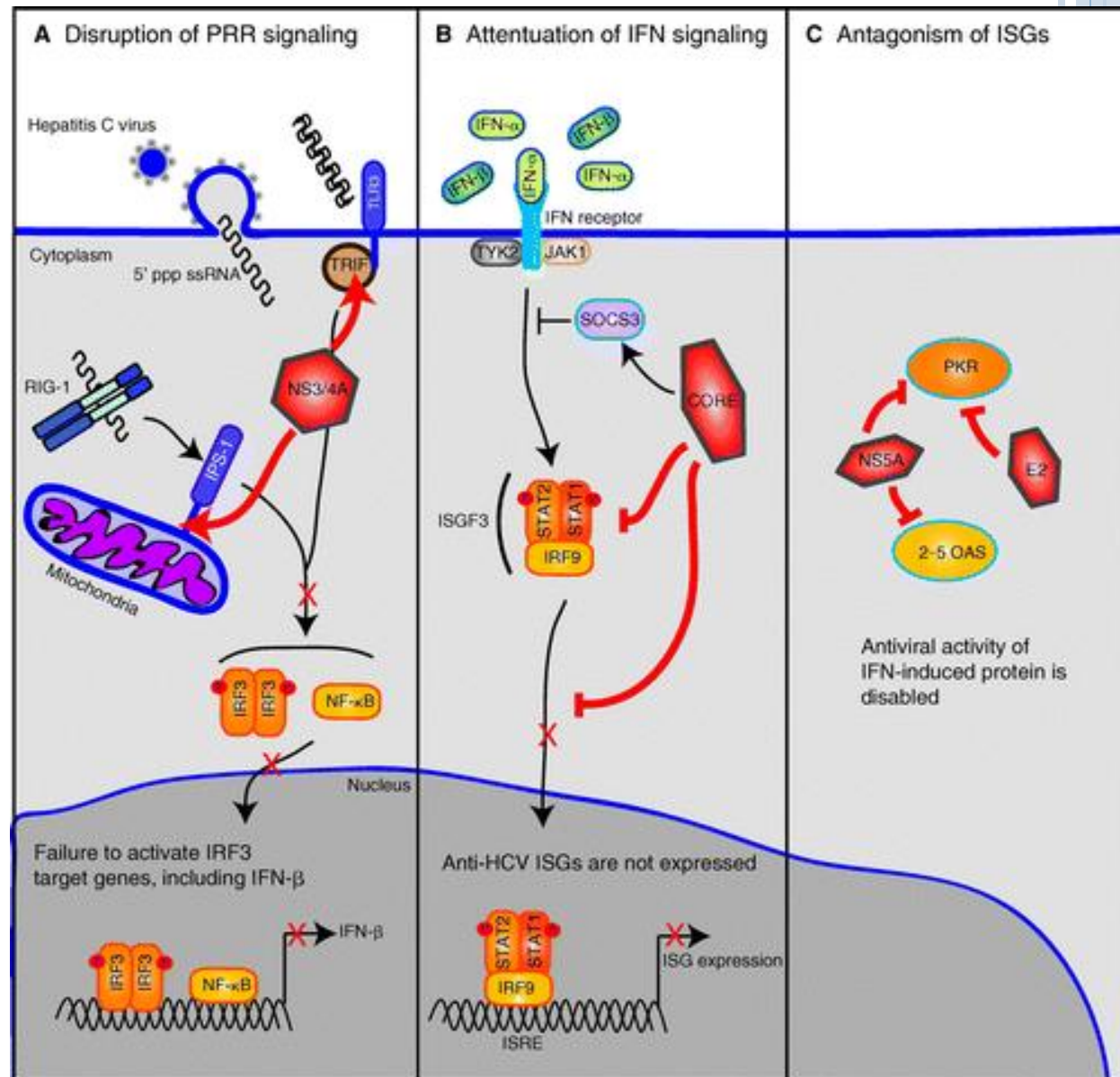
INDUCTION OF INNATE IMMUNITY IN PATIENTS

- IFN-induced genes interfere with viral replication directly:
 - Reducing protein synthesis by inhibiting initiation factors (PKR, ISG56)
 - Targeting of viral RNA (OAS, RNaseL)
- Innate responses can enhance or initiate adaptive responses
 - MHC I expression
 - Chemokine secretion and recruitment of responder cells



INNATE RECOGNITION OF HCV

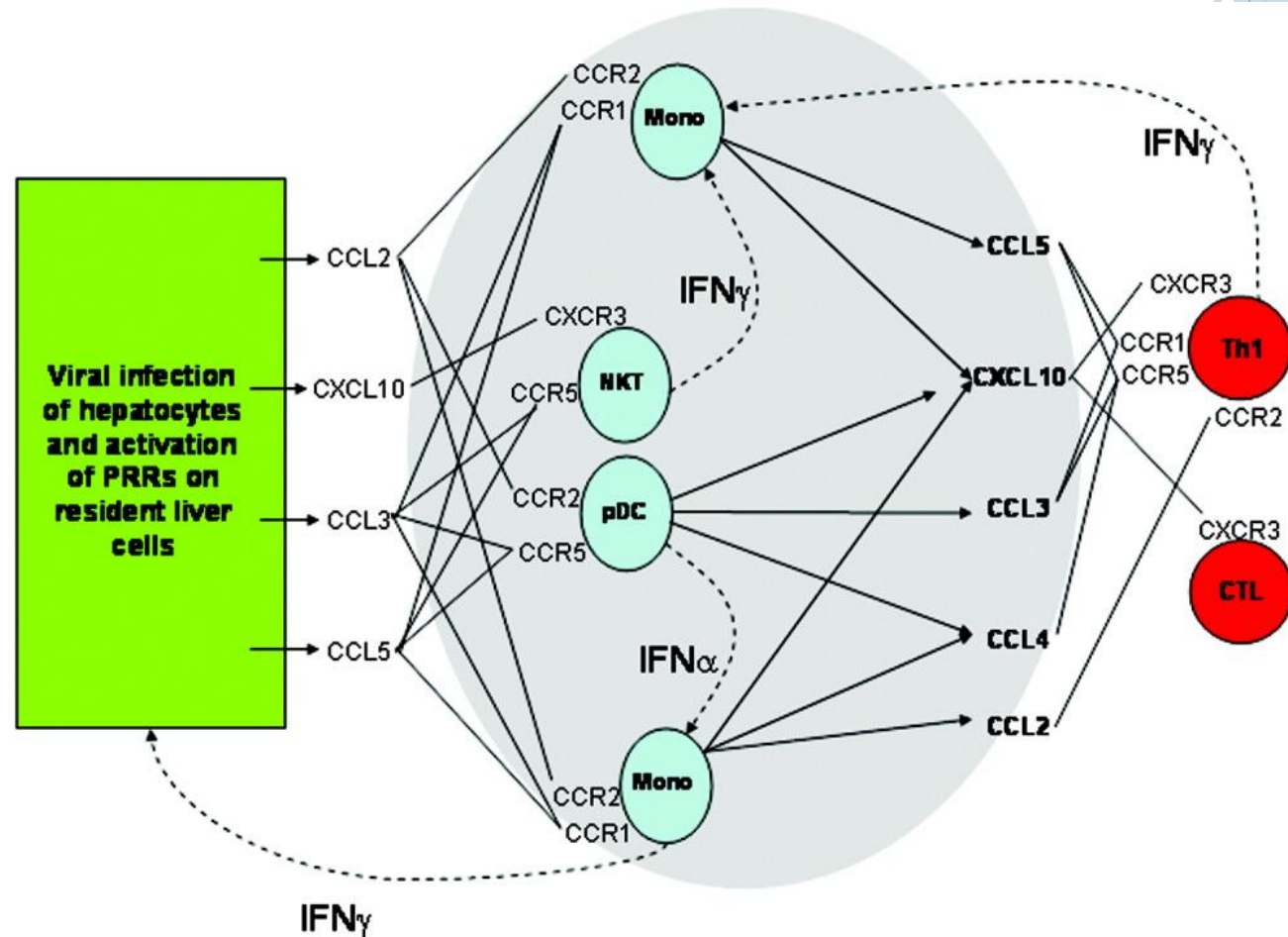
- The generation of dsRNA structures in HCV replication leads to recognition by multiple innate pathways
- HCV subverts these pathways by sequestering or cleaving key components of innate recognition
- The effects are both qualitative and quantitative on the ensuing innate response



Stacy M. Horner, Michael Gale. Journal of Interferon & Cytokine Research. September 2009, 29(9): 489-498

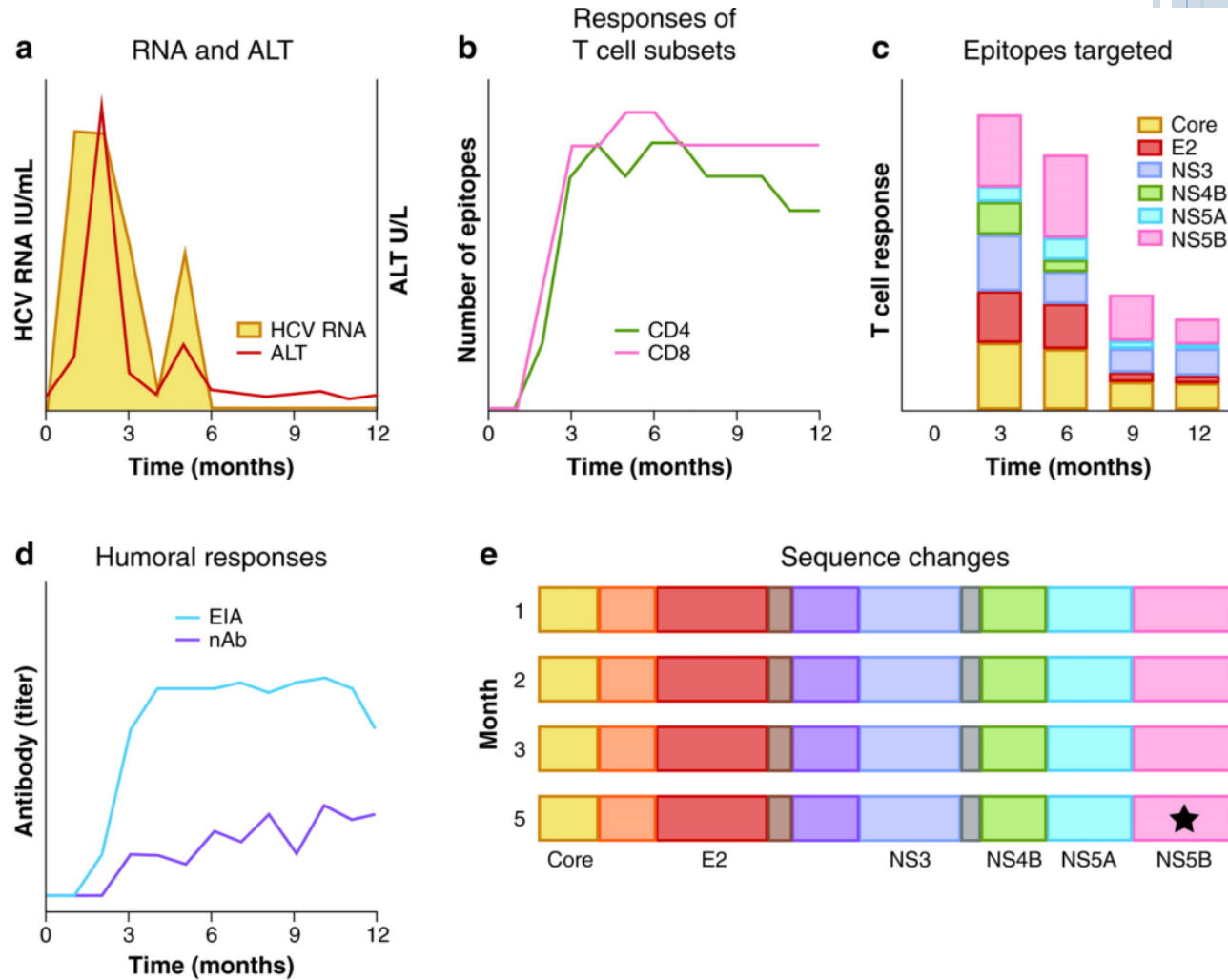
INNATE ACTIVATION OF ADAPTIVE RESPONSES

- The innate response results in the recruitment and “biasing” of key innate and adaptive cell types, including NK cells, NKT cells, antigen-presenting cells (monocytes/macrophages) and ultimately CD4 T cells that will orchestrate the adaptive response



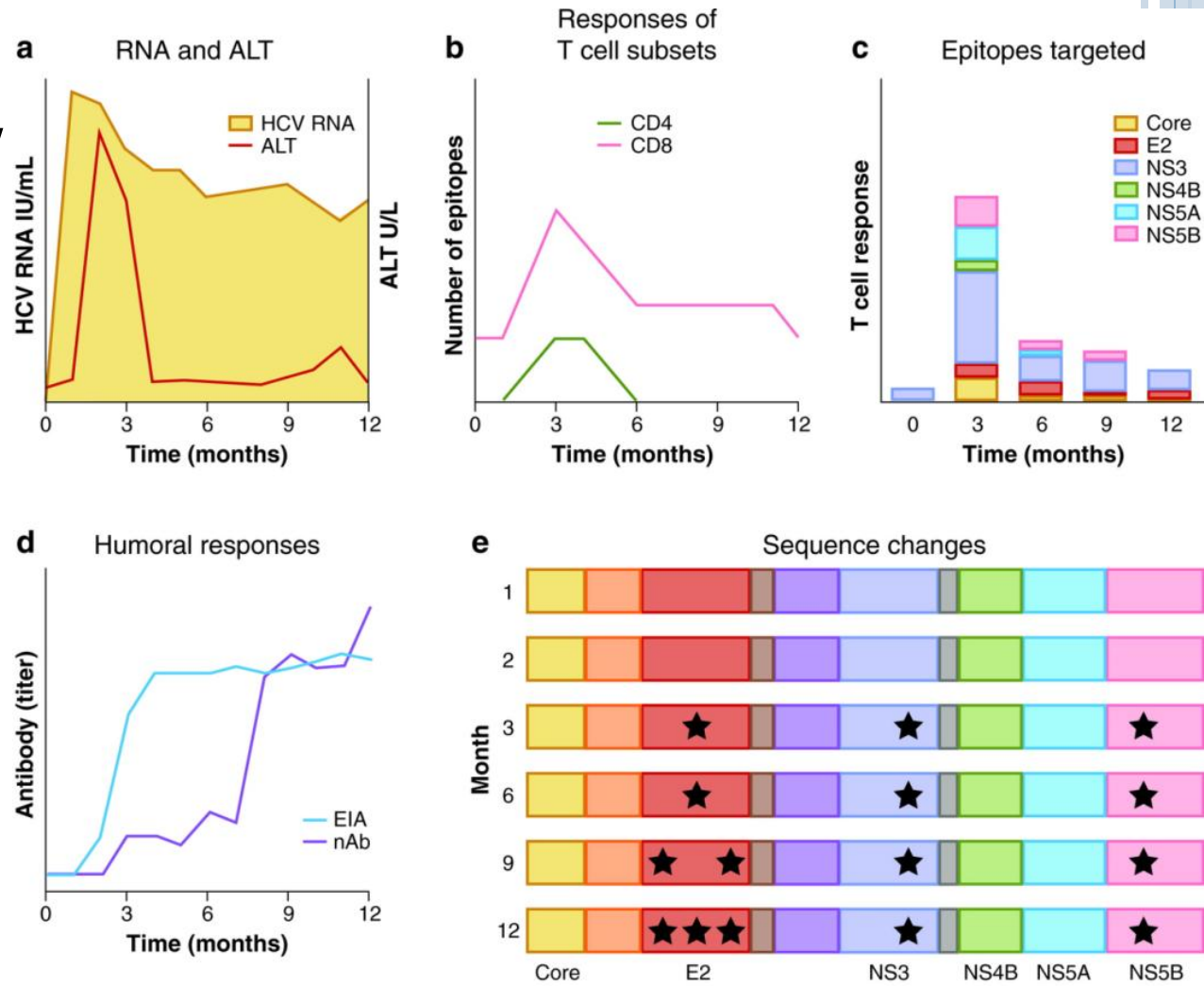
SUCCESSFUL HCV CONTROL (SUSTAINED VIROLOGICAL RESPONSE) IS MEDIATED BY ROBUST ADAPTIVE IMMUNITY

- Broad-based immunological repertoires (targeting multiple epitopes with diverse populations) control acute and prevent the development of chronic infections—particularly CD4 and CD8 cells (the role of antibody is controversial)

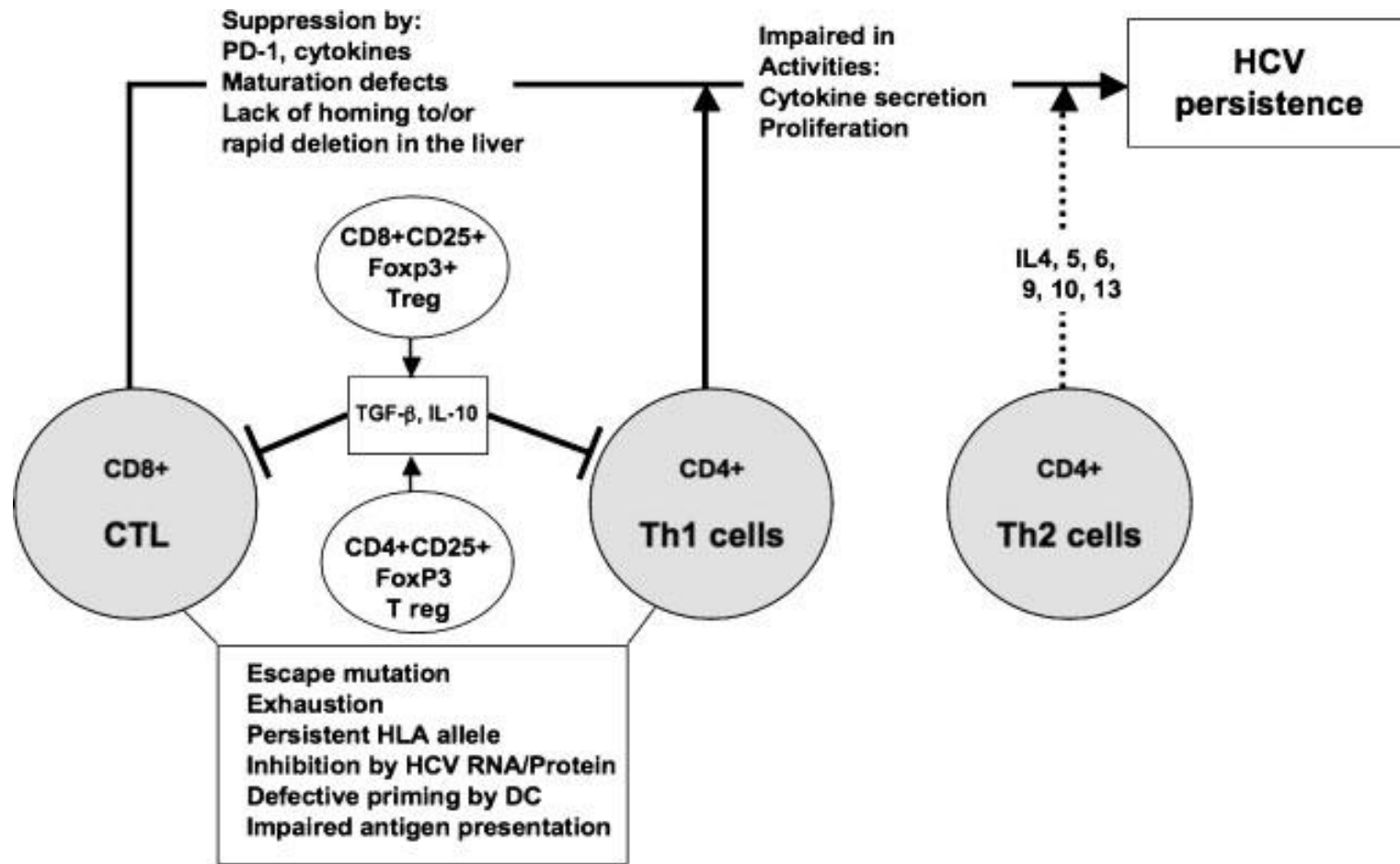


CHRONIC HCV INFECTIONS RESULT FROM POOR T CELL CONTROL, EPITOPE ESCAPE AND LIMITED REPERTOIRES

Limited TCR diversity, restricted epitope targets and dysfunctional T cell regulation result in weak T cell responses that are unable to avoid immunological escape



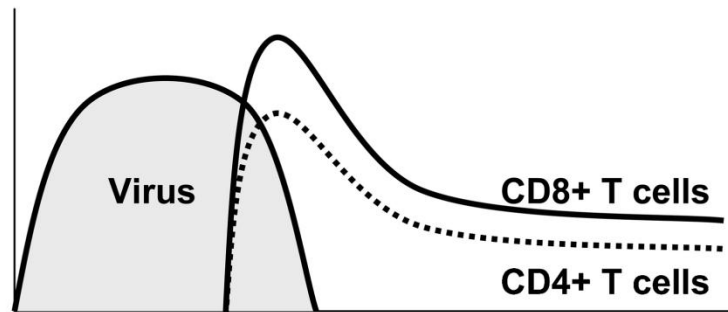
CHRONIC INFECTIONS AND IMMUNOSUPPRESSION



- Th2 biasing or immune senescence result in the downregulation of aggressive immunological control by CTL, providing the opportunity for viral escape and establishment of chronic infection

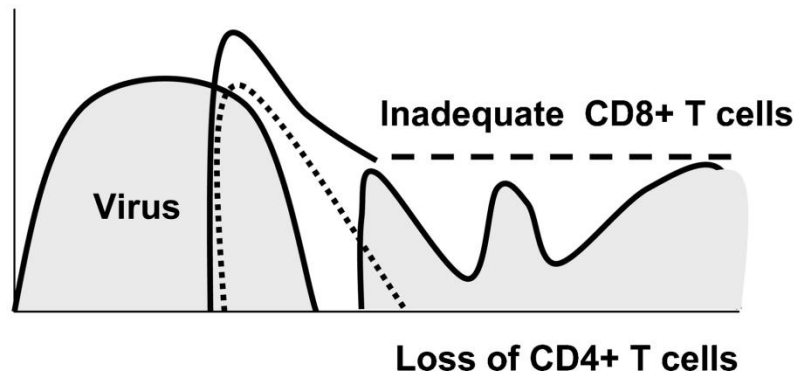
SUSTAINING AN EFFECTIVE CELLULAR RESPONSE IS MORE IMPORTANT THAN PEAK RESPONSE NUMBERS

A. Successful immune response



Long-lived memory

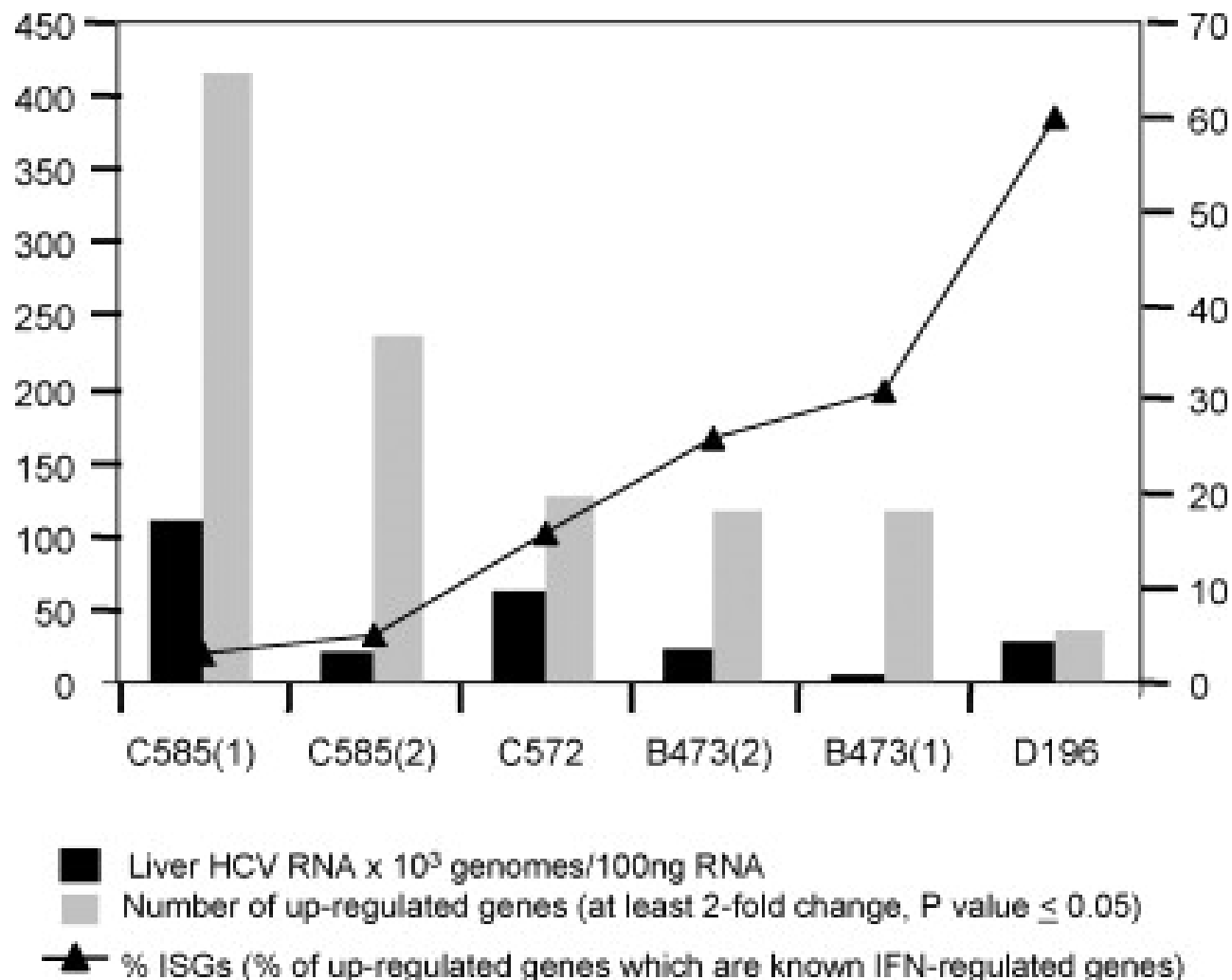
B. Unsuccessful immune response



Persistent viremia

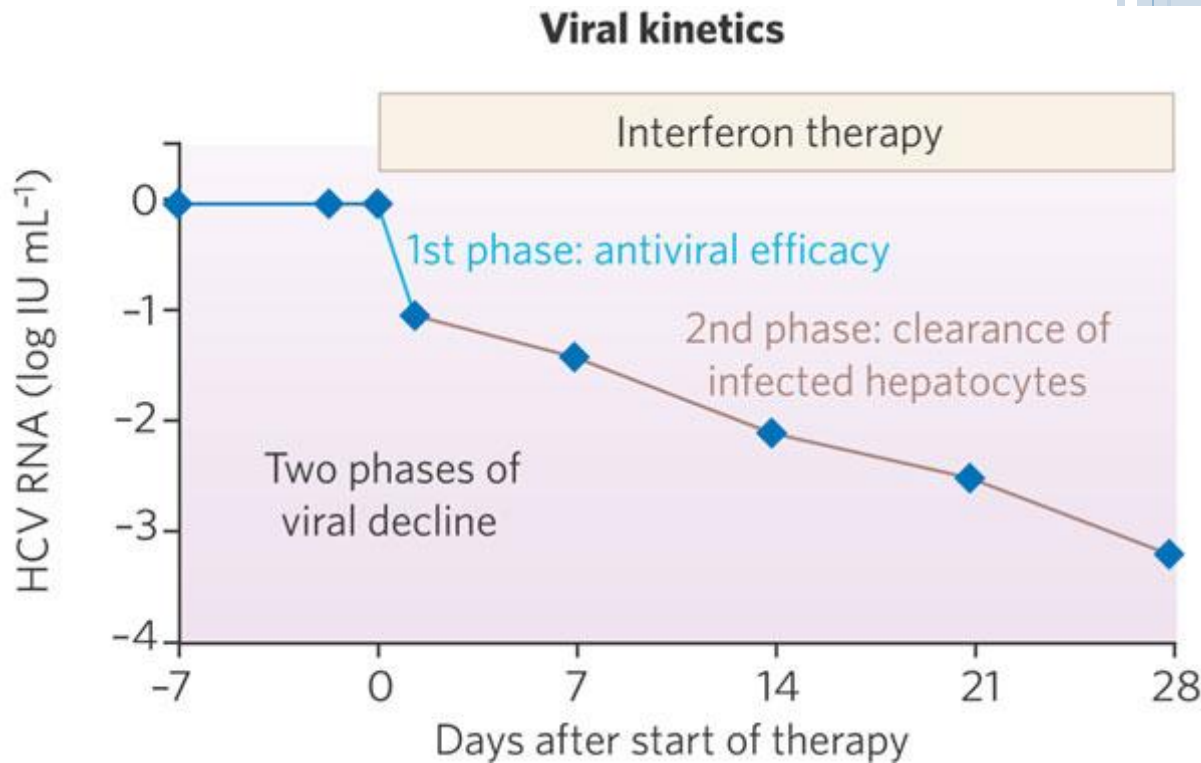


CONTROL OF ACUTE INFECTION CORRELATES WITH INTERFERON-INDUCED GENES



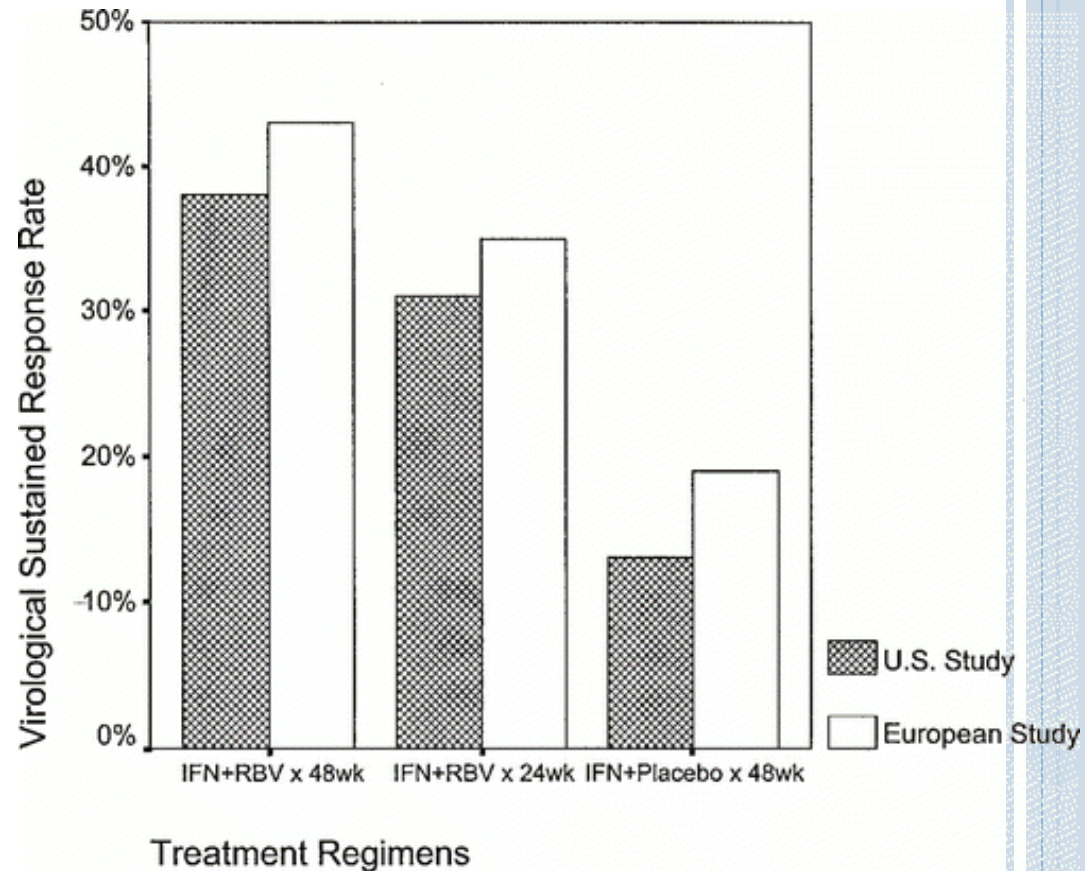
TREATMENT: TYPE I INTERFERON

- First therapy introduced for HCV
- Full mechanism of action unclear—presumably enhances the “normal” interferon response pathways
- Genotype of virus, low baseline levels of HCV RNA and stage of infection are the strongest correlates of efficacy
- Suggestions that immunomodulation may play a role and that high dose-interferon may overcome some of the “regulatory” negative feedback loops active in the infected host
- Overall, the specific mechanism has not been clearly demonstrated biologically



COMBINATION THERAPY IS SIGNIFICANTLY MORE EFFECTIVE

- Interferon alone only yields a 20-25% response rate following a 12-18 month course
- Combination therapy with the “broad based” antiviral ribavirin results in 40% of individuals with SVR (30% genotype 1, 65% genotype 2 or 3)

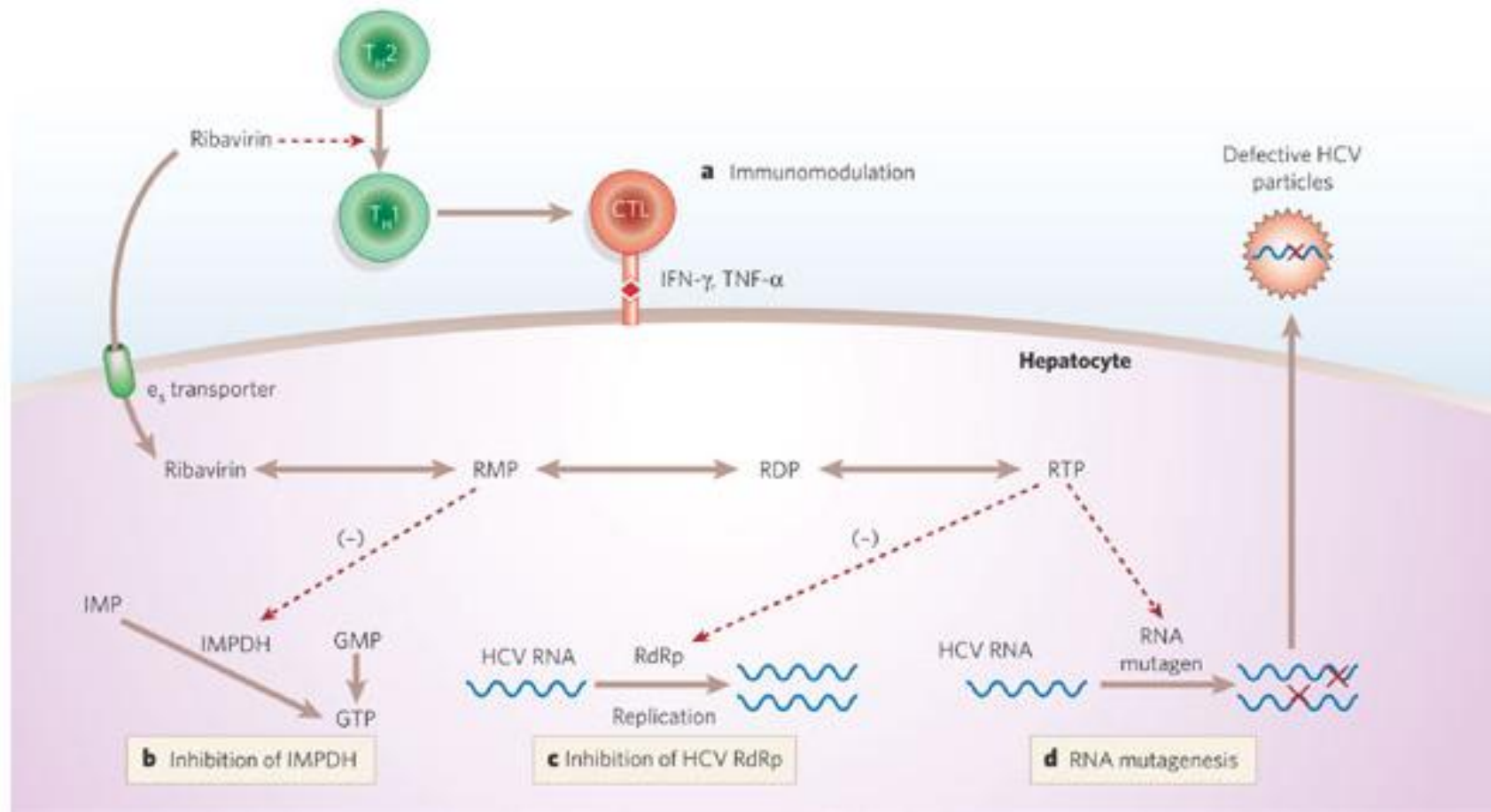


HOW DOES RIBAVIRIN WORK AGAINST HCV?

- Ribavirin was initially designed as a nucleoside analog and developed as an anti-influenza drug, but failed to receive FDA approval or show significant efficacy in humans
- It has been used to treat hemorrhagic fevers, RSV and is again under consideration as combination therapy for influenza
- Proposed Mechanisms:
 - 1) Immunomodulatory properties
 - 2) Inhibition of the inosine monophosphate dehydrogenase (IMPDH)
 - 3) Direct inhibition of the HCV-encoded NS5B RNA polymerase
 - 4) Induction of lethal mutagenesis
 - 5) Modulation of interferon-stimulated gene (ISG) expression



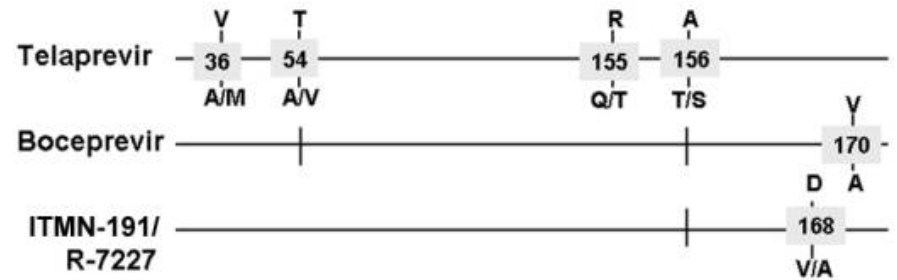
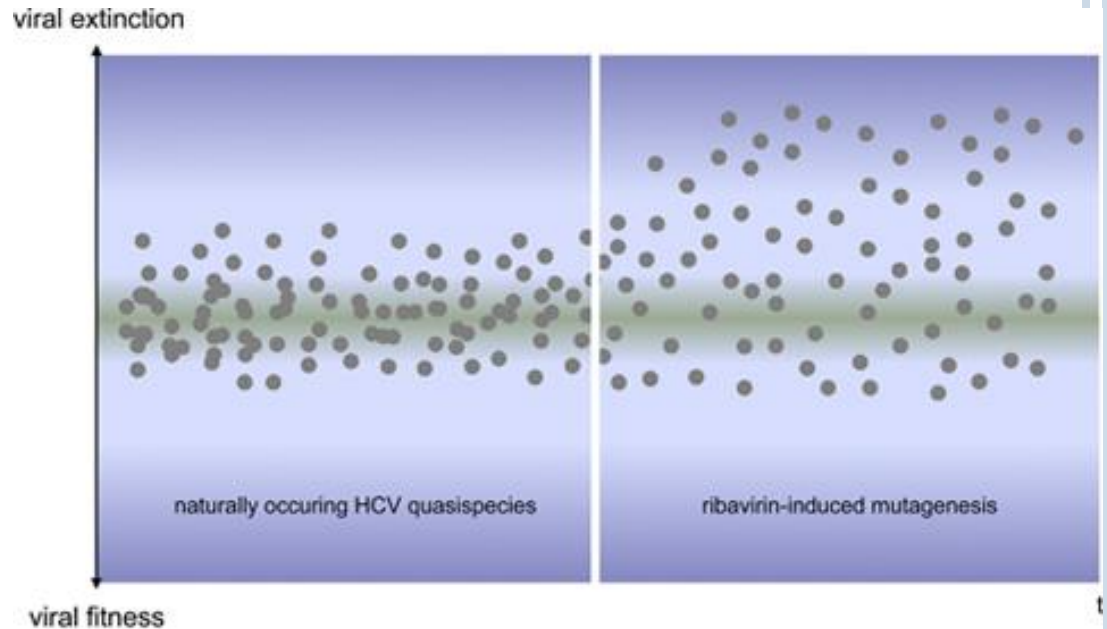
POSSIBLE MECHANISMS FOR RIBAVIRIN MODE OF ACTION



WHAT DATA WOULD HELP RESOLVE RIBAVIRIN'S MECHANISM?

Interferon reduces viral production-- given the proposed mechanisms, how should ribavirin work?

- 1) Immunomodulatory properties—
Should act independently of interferon
- 2) Inhibition of the inosine monophosphate dehydrogenase (IMPDH)—**Should reduce viral production, be guanosine dependent**
- 3) Direct inhibition of the HCV-encoded NS5B RNA polymerase—**Should reduce viral production, put pressure on polymerase to mutate**
- 4) Induction of lethal mutagenesis—**Viral production maintained, infected cell number maintained (clearance by decay), new cells infected at a lower rate**
- 5) Modulation of interferon-stimulated gene (ISG) expression—**Direct antiviral effects like interferon, should shift ISG expression from negative feedback pathways and be synergistic with poor interferon responders.**

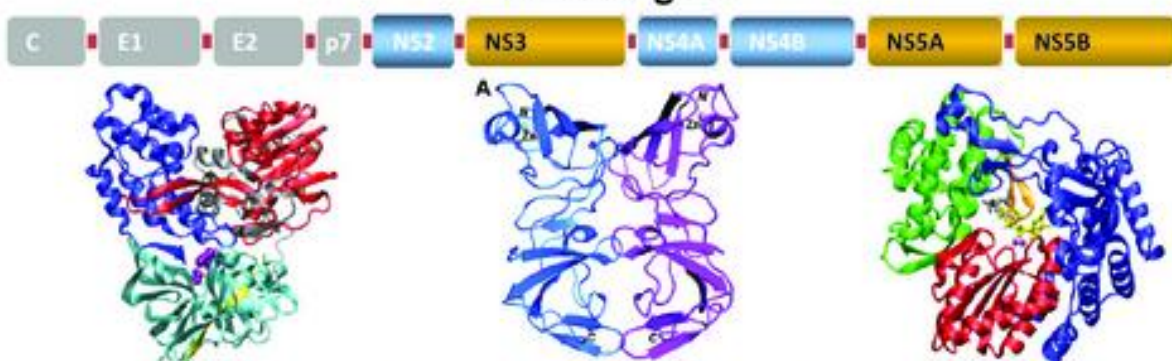
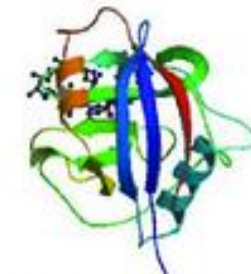


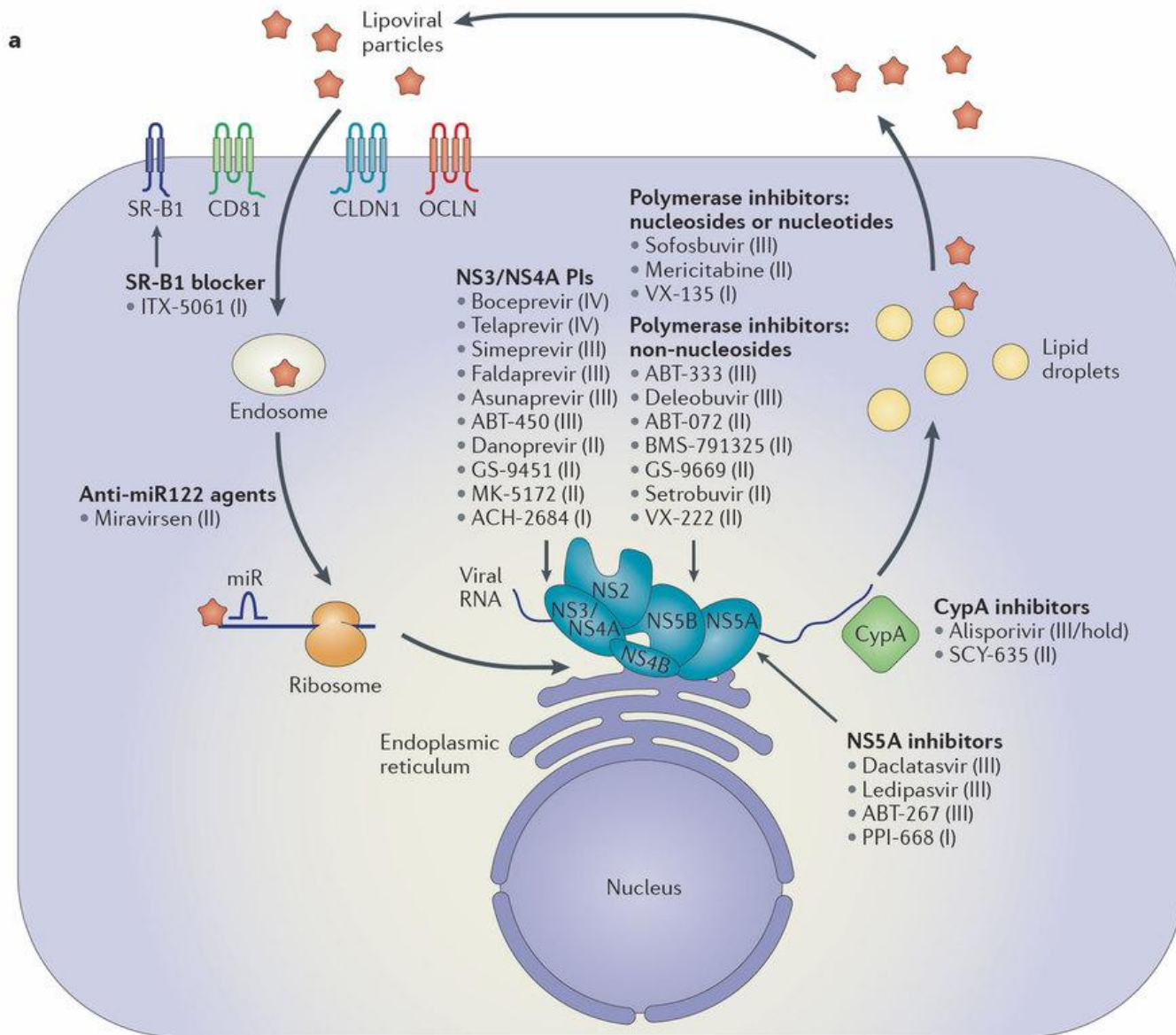
DETERMINING AN ANTIVIRAL TREATMENT'S MODE OF ACTION

- Biological *in vitro* experiments with HCV have been difficult to perform as a result of the limited nature of developed culture systems
- Alternative drugs that perform a single “ribavirin function” do not recapitulate ribavirin efficacy, suggesting that multiple pathways may be acting together
- Biological mechanisms can often seem plausible, but can be difficult to prove conclusively that they play an important role (particularly when the drug is “reverse engineered” to the pathogen)
- Mathematical modeling from real infection data provides a compelling argument for the viral life cycle stage(s) that might be affected



NEW DRUG TREATMENTS FOR HCV

Viral targets			Host targets
			
NS3	NS5A	NS5B	Cyclophilin A
The NS3/4A serine protease	Multifunctional phosphoprotein, component of the HCV-RNA replication complex	RNA-dependent RNA polymerase	Host protein interacting with NS5A and the NS5B
Boceprevir Telaprevir ABT-450/r, ACH-1625 Asunaprevir, TMC-435 (Simeprevir), BI-201335 Danoprevir/r, GS-9451 MK-5172	Daclatasvir GS-5885 ABT-267 PPI-668 MK	<u>Nucleos(t)ide analogue</u> GS-7977 (Sofosbuvir), Mericitabine, IDX-184 <u>Non-nucleoside analogue</u> BI-207127, ABT-333 ABT-072, BMS-791325 Tegobuvir, Setrobuvir VX-222, Filibuvir	Alisporivir SCY-635



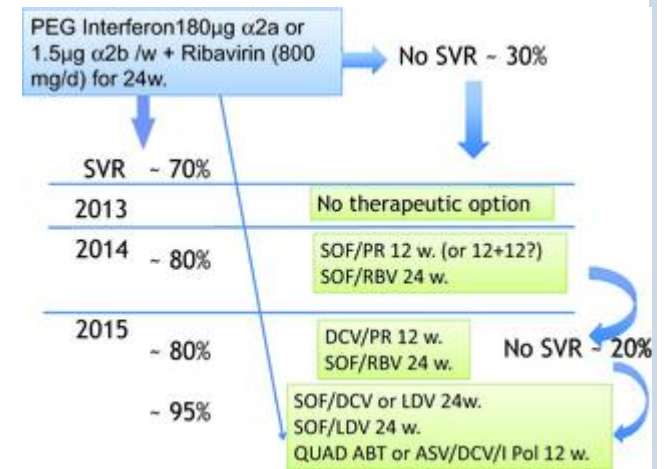
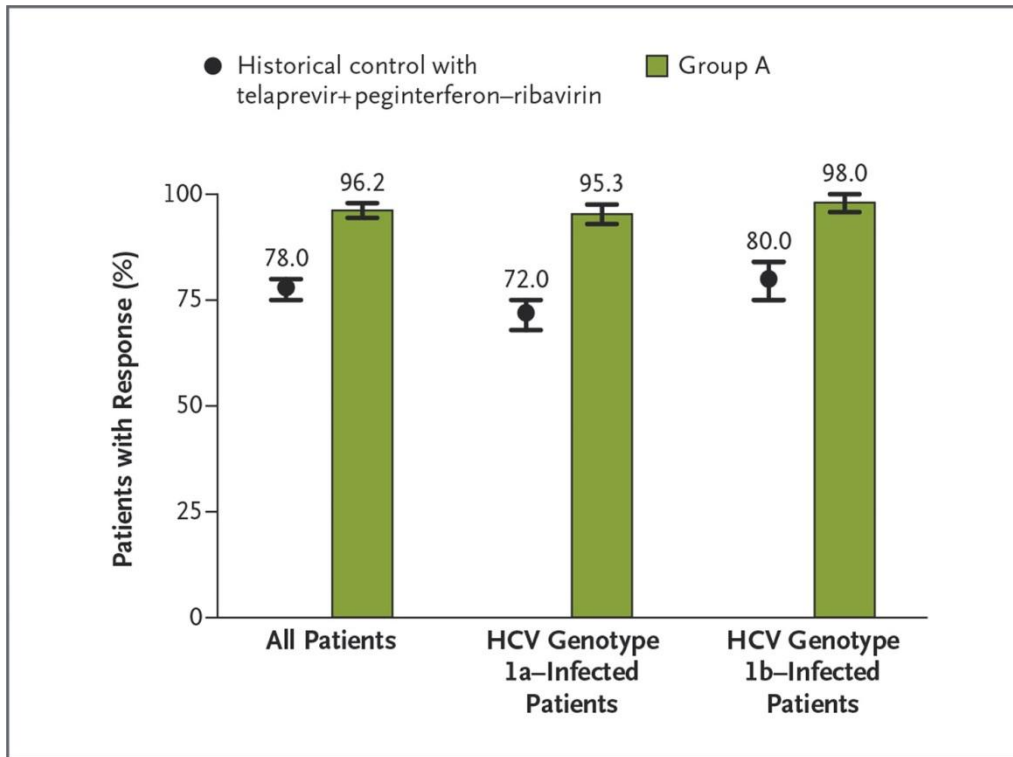
Novel therapies for hepatitis C — one pill fits all?

Michael P. Manns

& Thomas von Hahn

Nature Reviews Drug Discovery 12, 595–610 (2013) doi:10.1038/nrd4050

Rates of Sustained Virologic Response among All Patients and According to HCV Genotype in the Historical Control Group and in Group A.

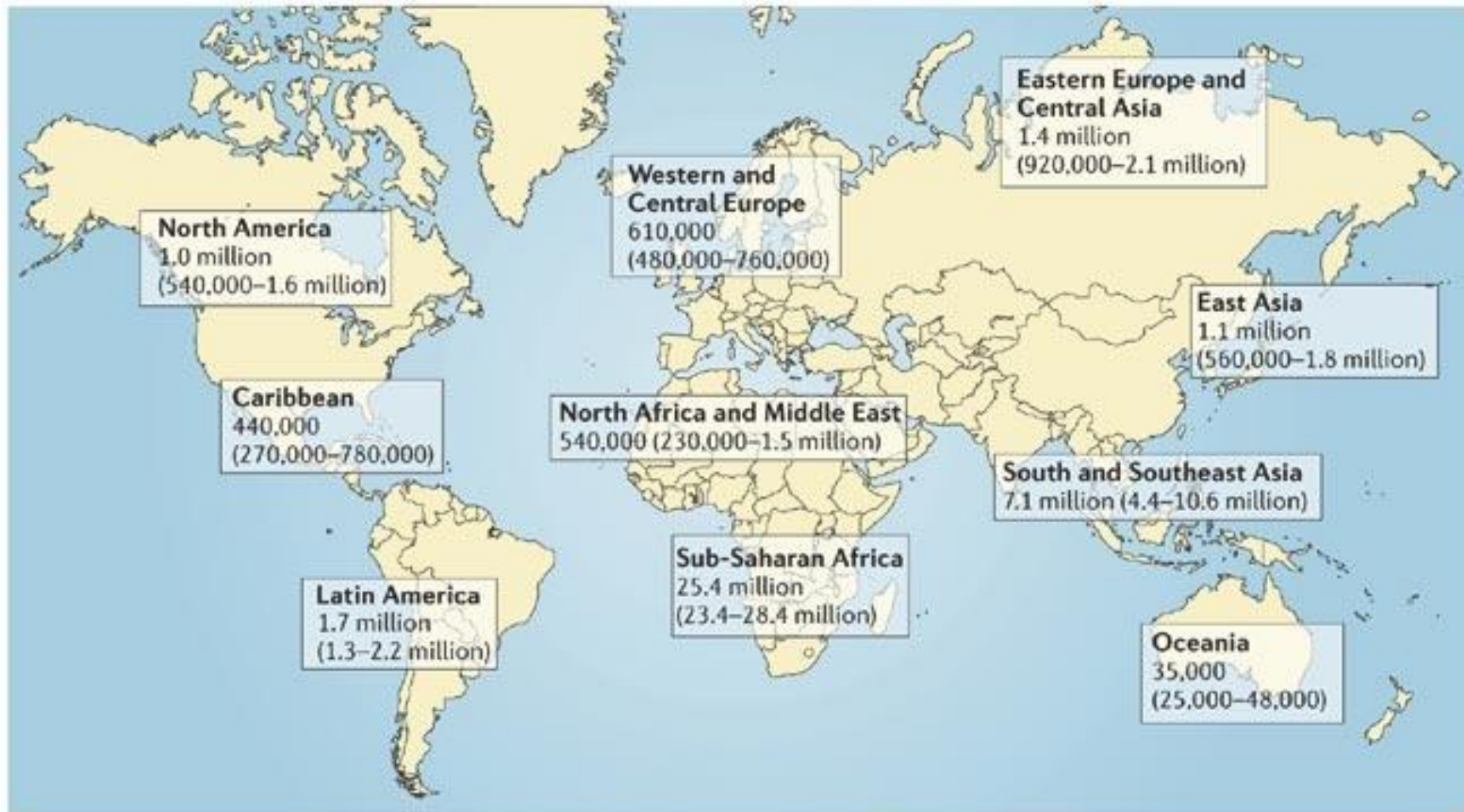


FELD JJ ET AL. N ENGL J MED 2014;370:1594-1603.

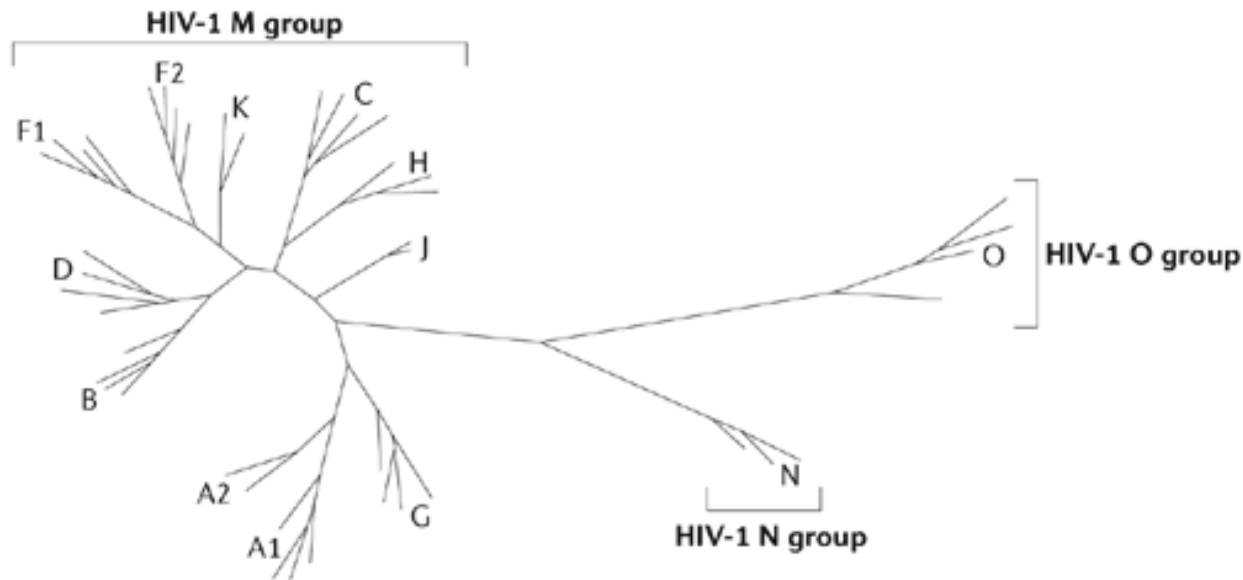


The NEW ENGLAND
JOURNAL of MEDICINE

PREVALENCE OF HIV INFECTION



GENETIC DIVERSITY OF HIV-1

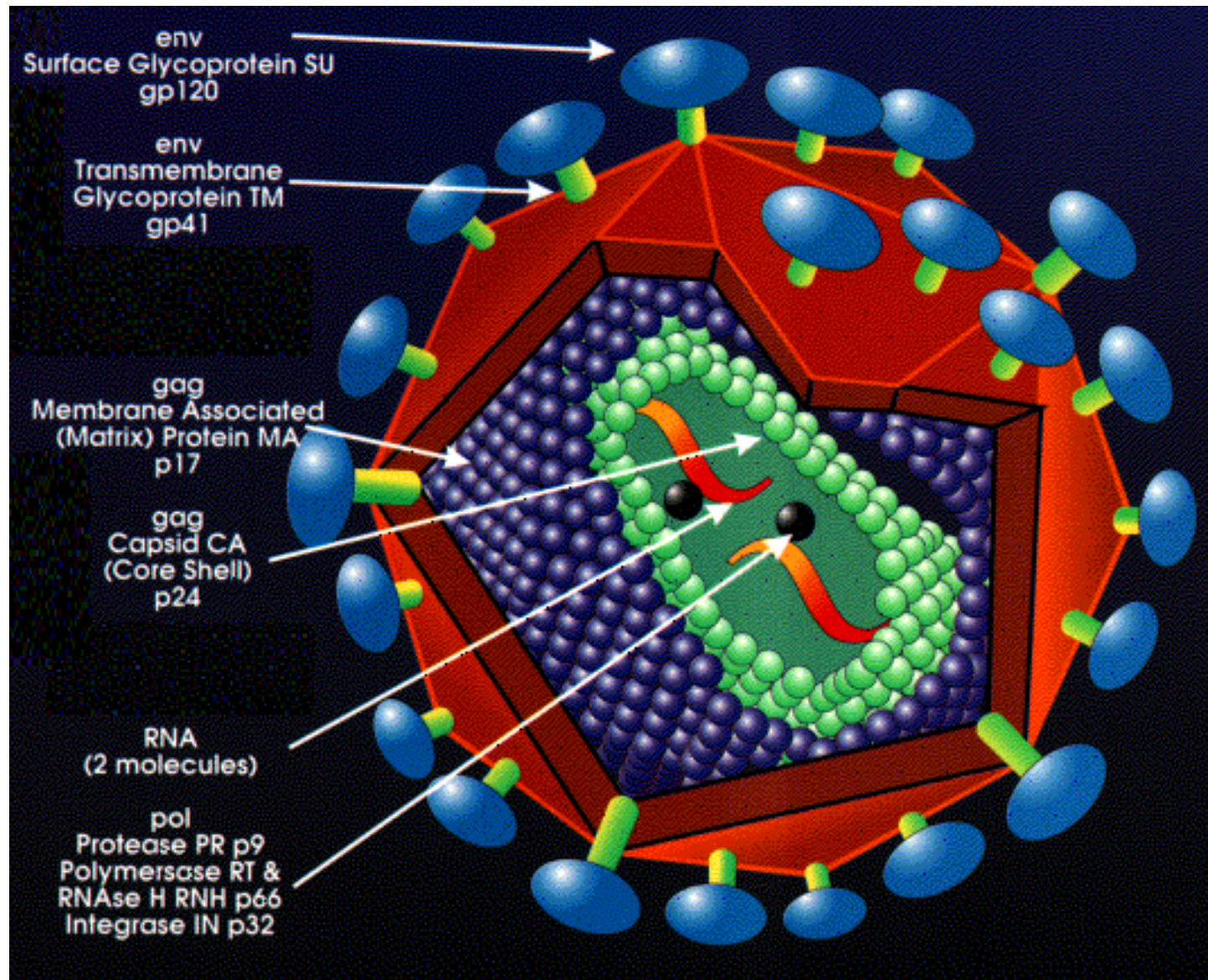


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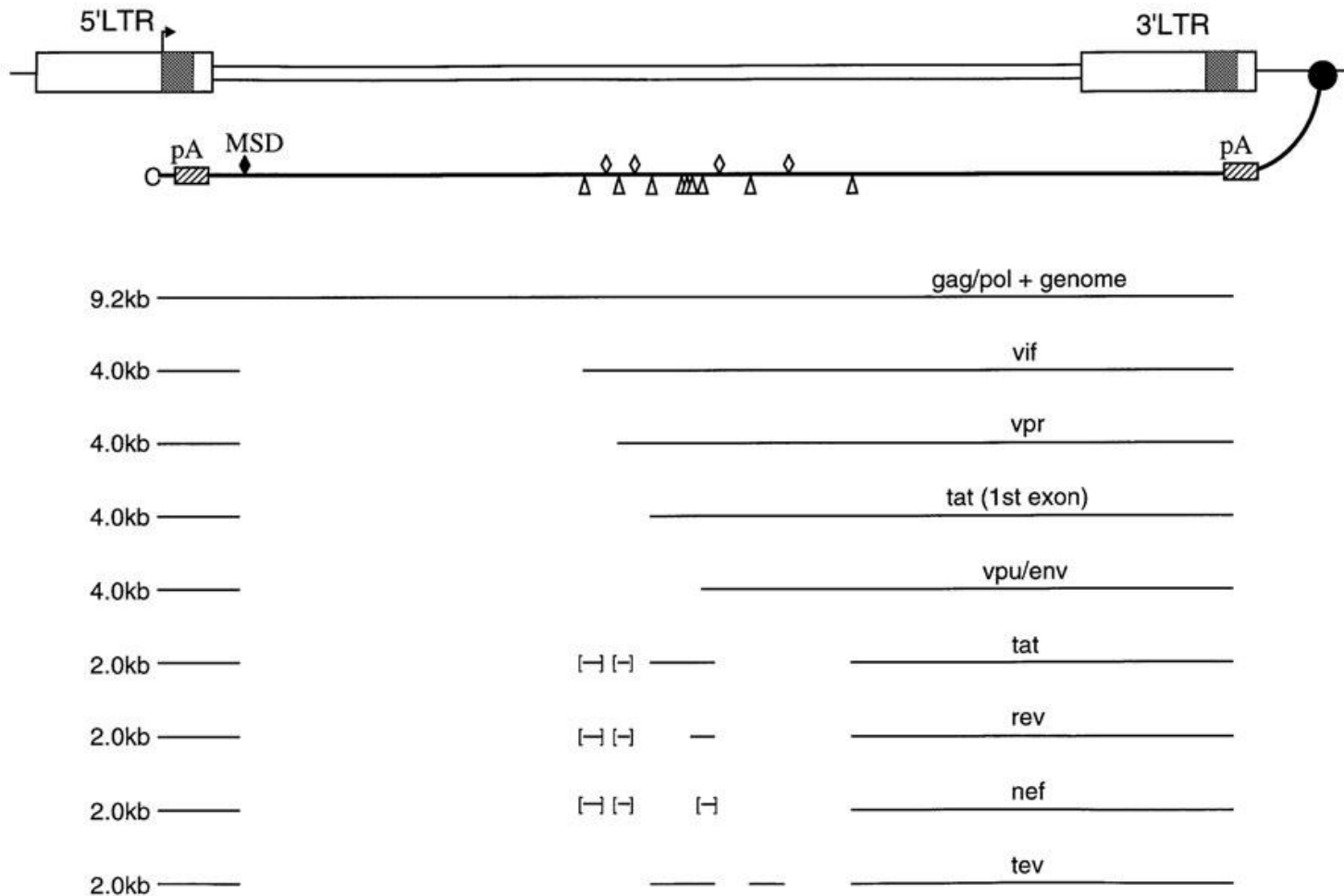
- Within HIV-1, a large sequence diversity exists with viral clades being geographically isolated
- Several studies have suggested that the clades have different biological characteristics, including disease pathogenicity and transmissibility



VIRION STRUCTURE

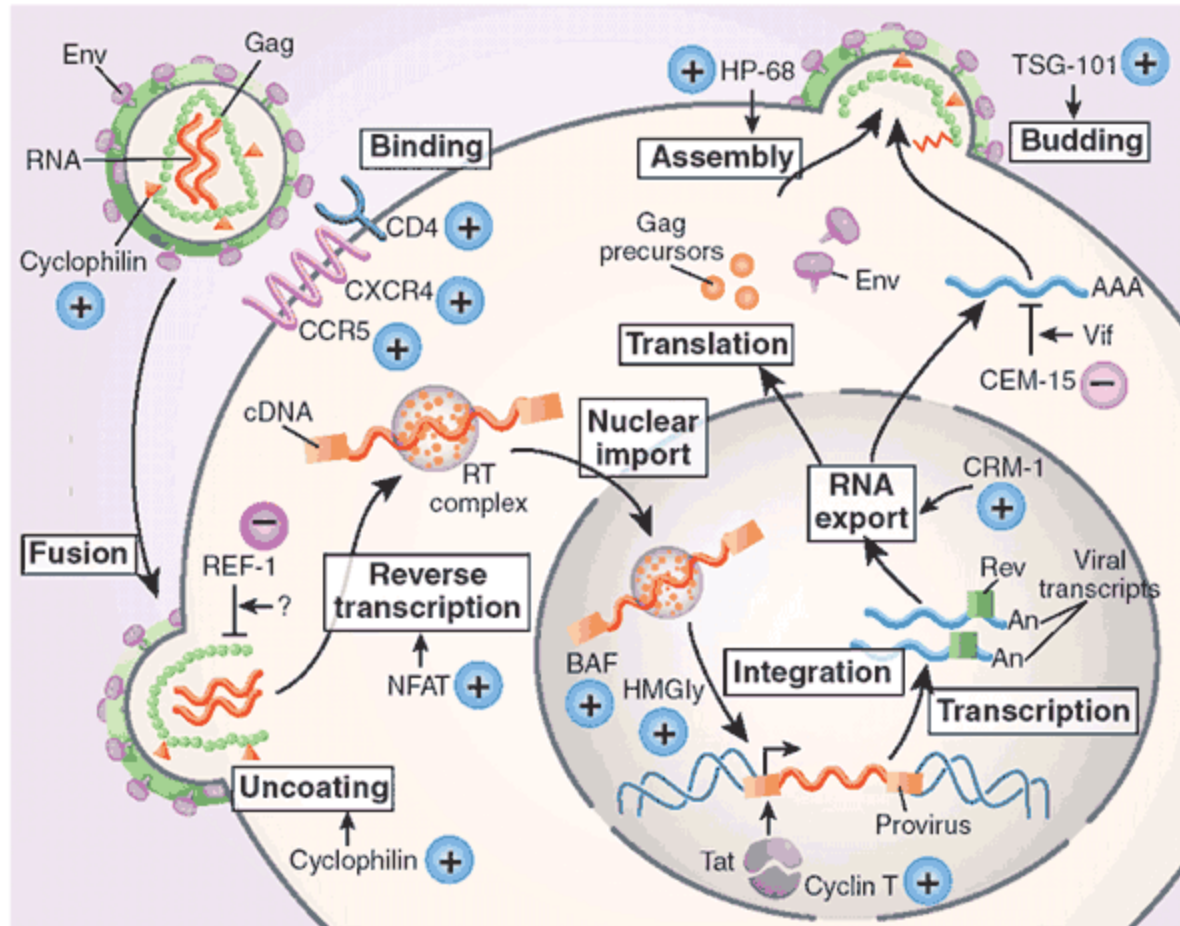


SINGLE STRANDED GENOME, MULTIPLE MESSAGES FROM ALTERNATIVE SPLICING



VIRAL LIFE CYCLE

- As a retrovirus, HIV replicates by making a DNA copy of itself that is inserted into the host genome
- Thus, an infected cell can become a stable reservoir for the long term production of viral particles

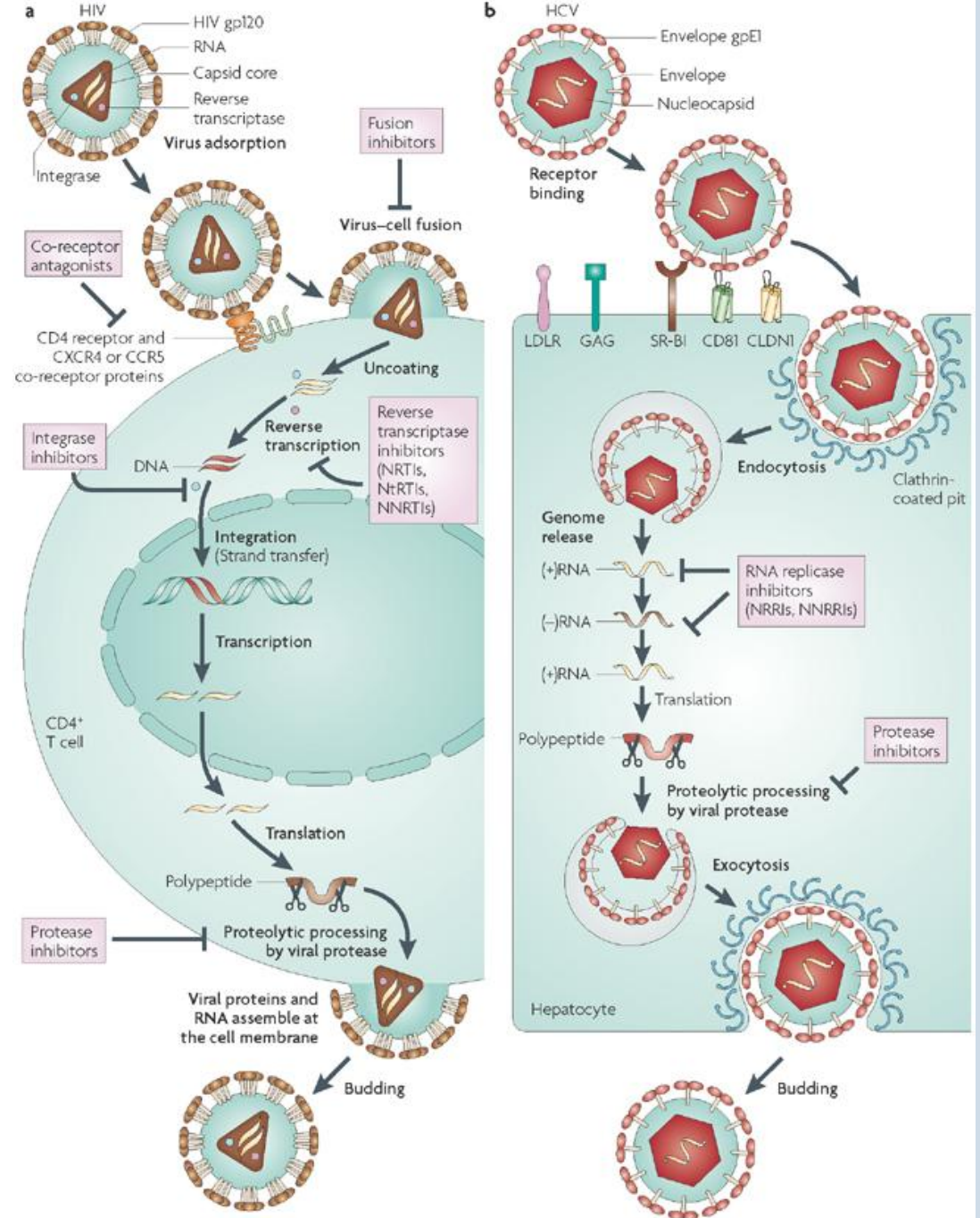


COMPARISON OF HIV AND HCV

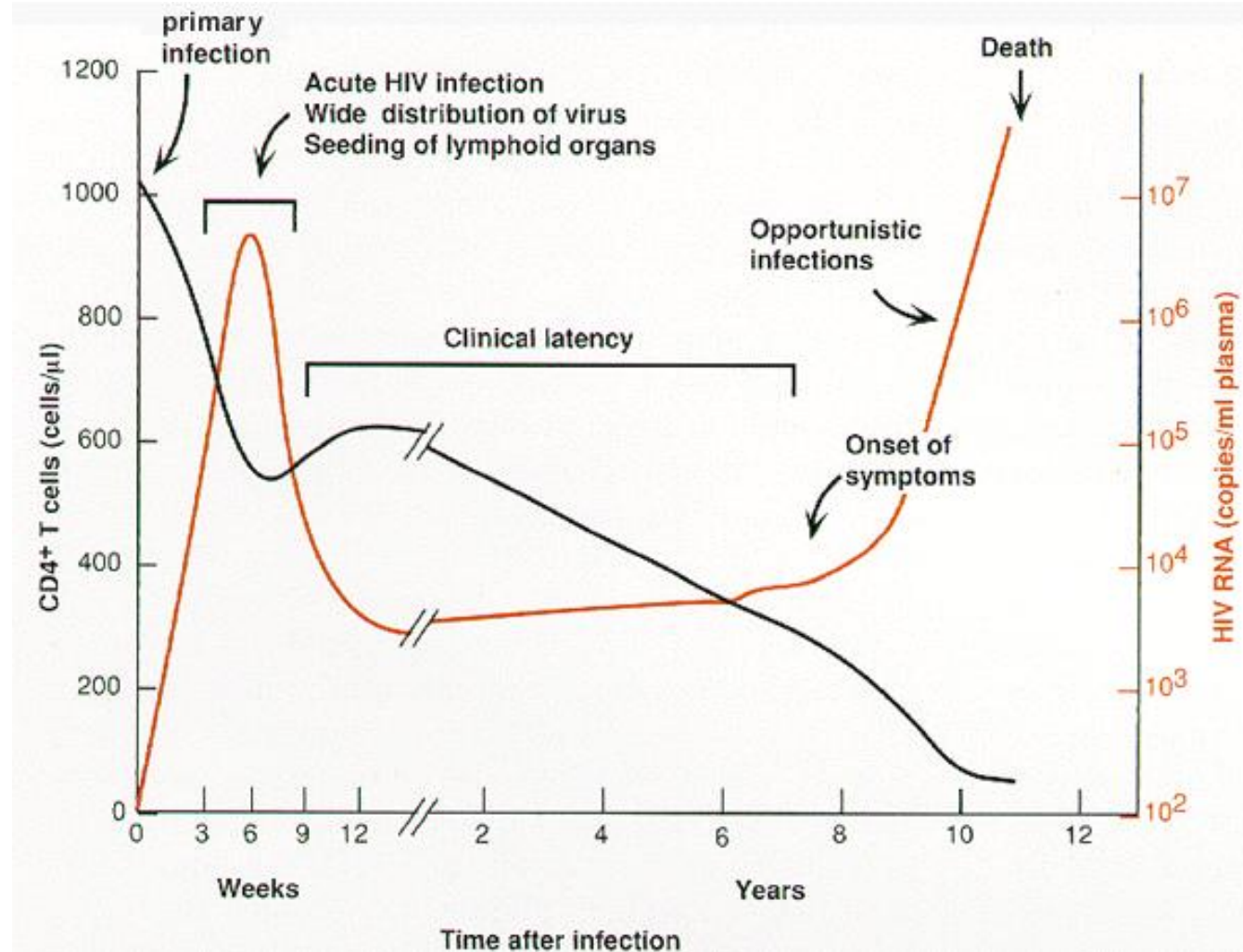
HIV and HCV both produce chronic infections, but are biologically very different viruses

HIV has a DNA intermediate that become heritably integrated

HCV is a purely RNA virus

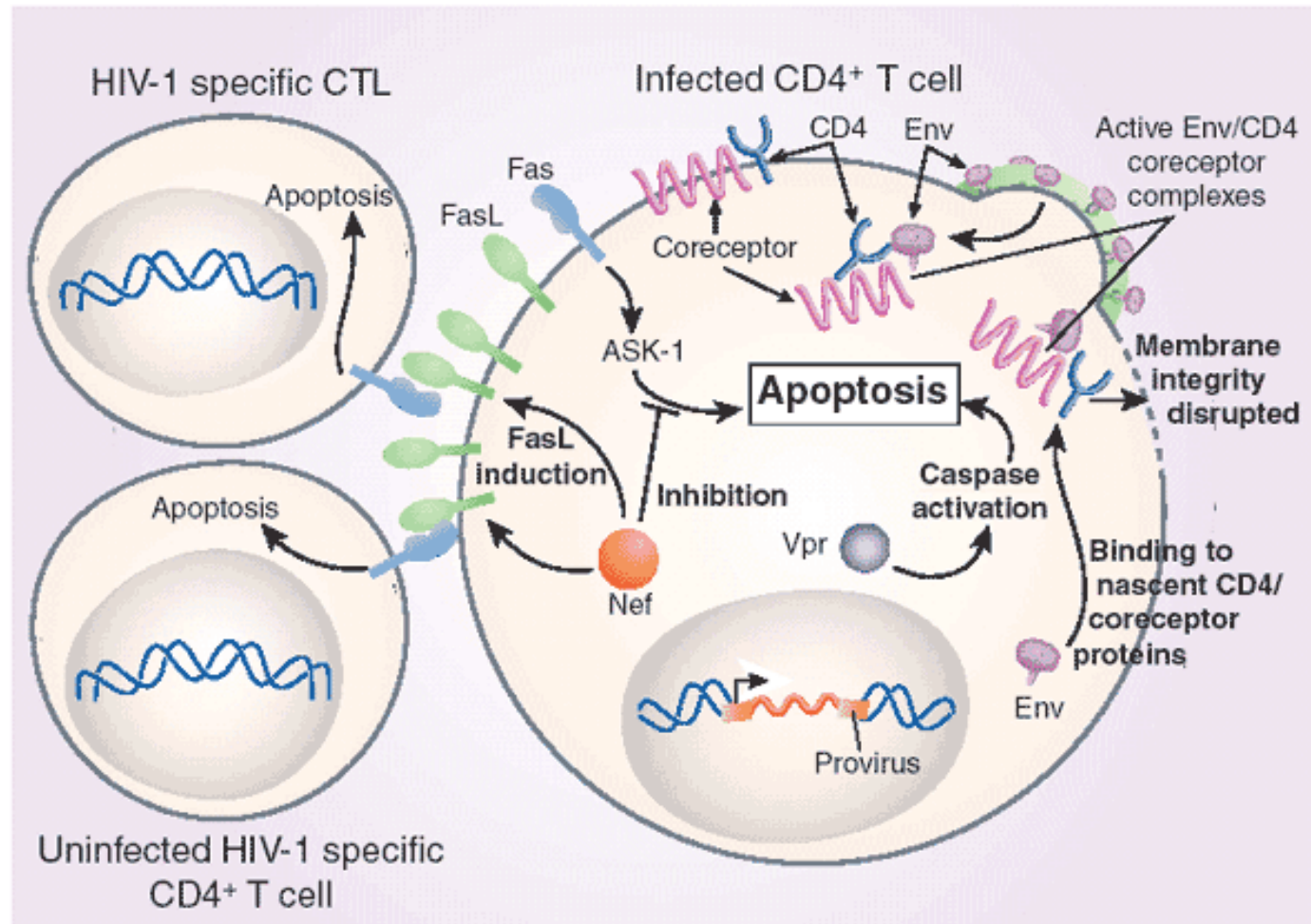


CLINICAL COURSE OF INFECTION



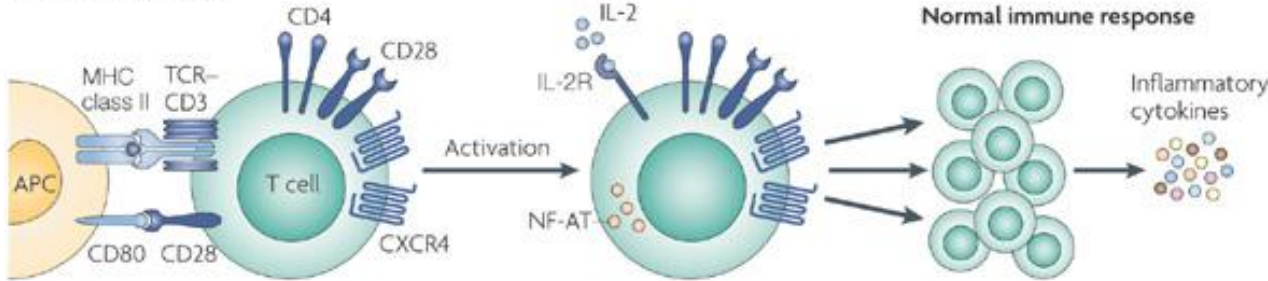
MECHANISMS OF CYTOPATHOGENICITY

- Viral envelope fusogenicity (ER compromised)
- Vpr activates caspases
- Nef contributes indirectly to apoptosis via FasL

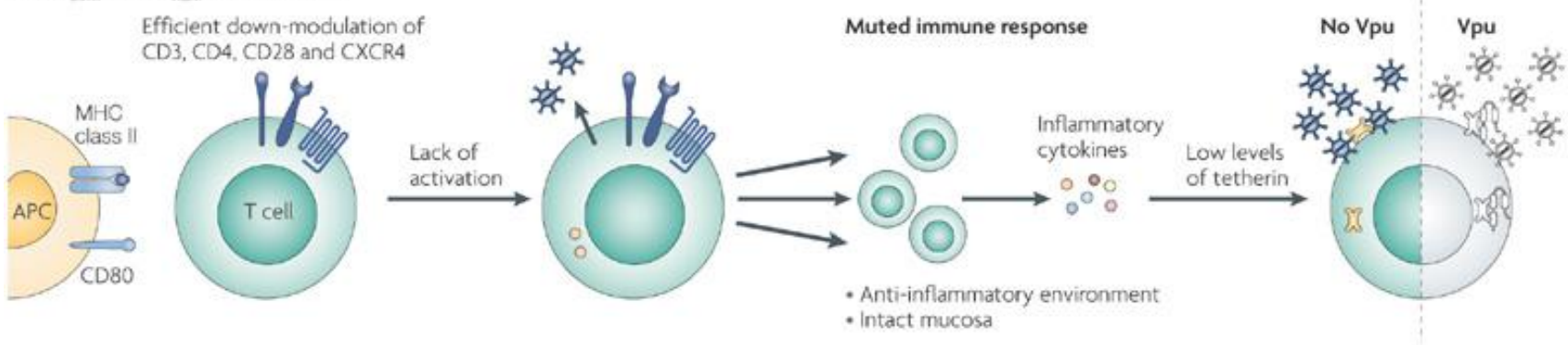


MECHANISMS OF IMMUNE DYSREGULATION

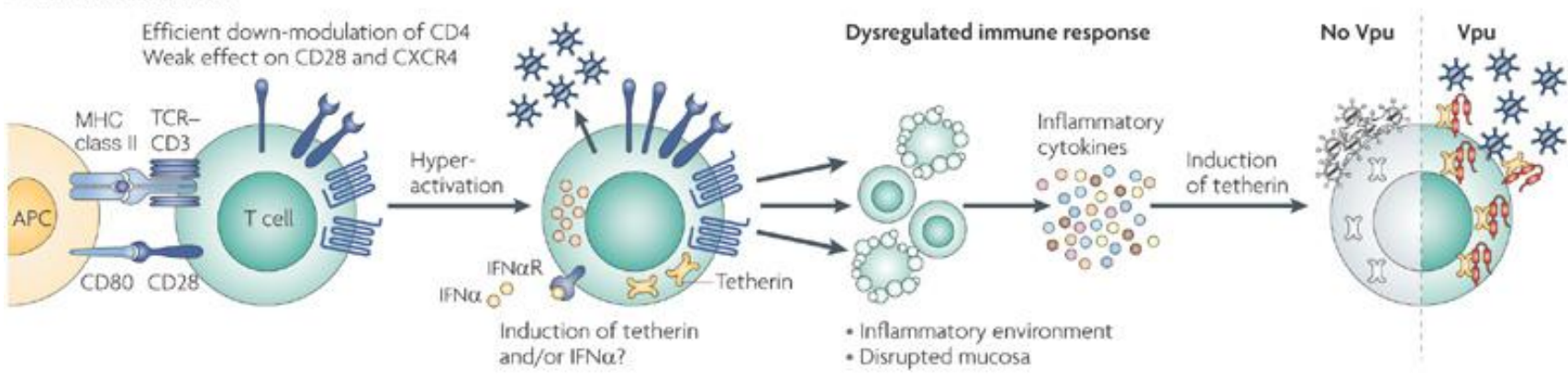
a Uninfected T cell



b SIV_{SMM}- or SIV_{AGM}-infected T cell



c HIV-1-infected T cell



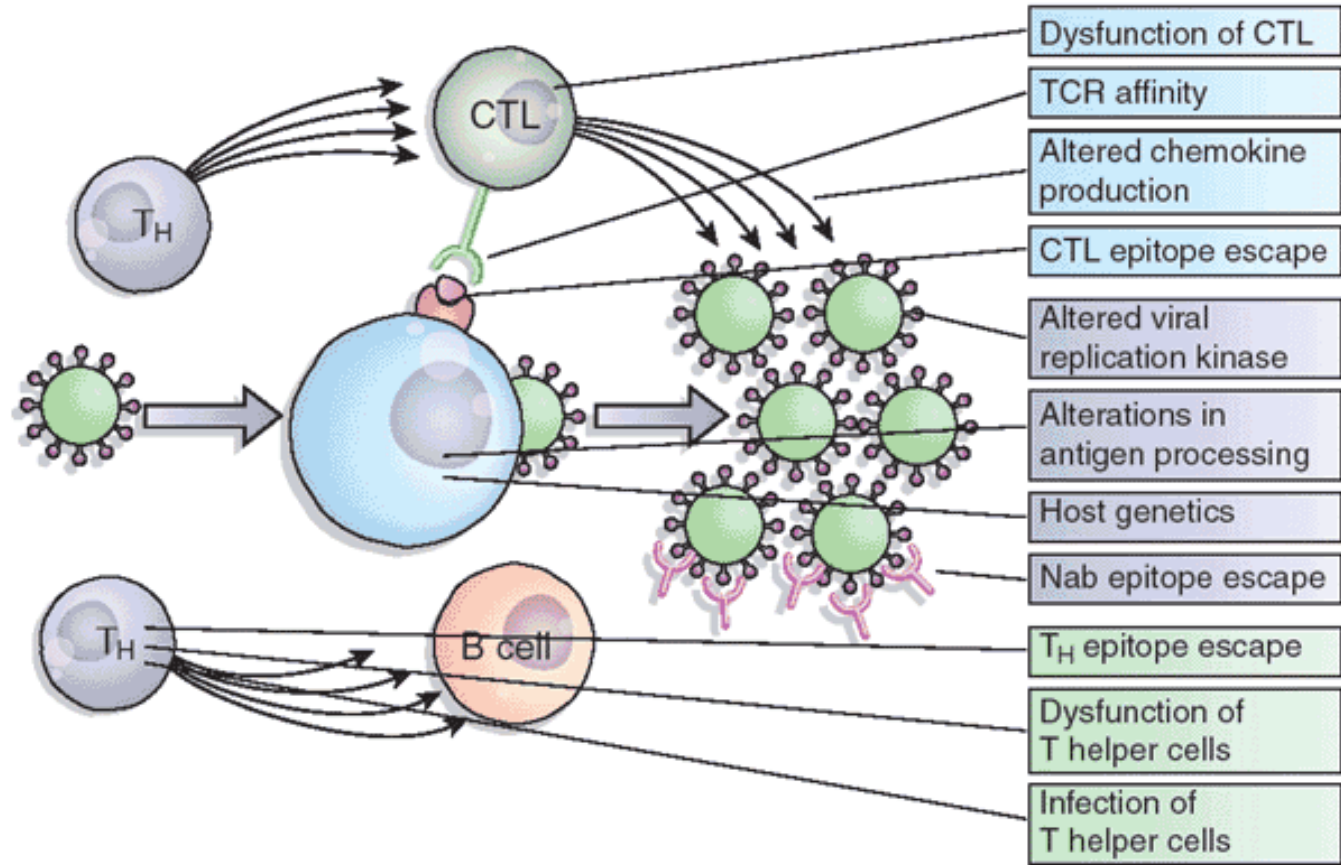
WHAT MAKES HIV LETHAL?

Infections		Malignancies
Parasites	<i>Toxoplasma</i> spp. <i>Cryptosporidium</i> spp. <i>Leishmania</i> spp. <i>Microsporidium</i> spp.	Kaposi's sarcoma - HHV8 Non-Hodgkin's lymphoma, including EBV-positive Burkitt's lymphoma Primary lymphoma of the brain
Intracellular bacteria	<i>Mycobacterium tuberculosis</i> <i>Mycobacterium avium intracellulare</i> <i>Salmonella</i> spp.	
Fungi	<i>Pneumocystis carinii</i> <i>Cryptococcus neoformans</i> <i>Candida</i> spp. <i>Histoplasma capsulatum</i> <i>Coccidioides immitis</i>	
Viruses	Herpes simplex Cytomegalovirus Varicella zoster	

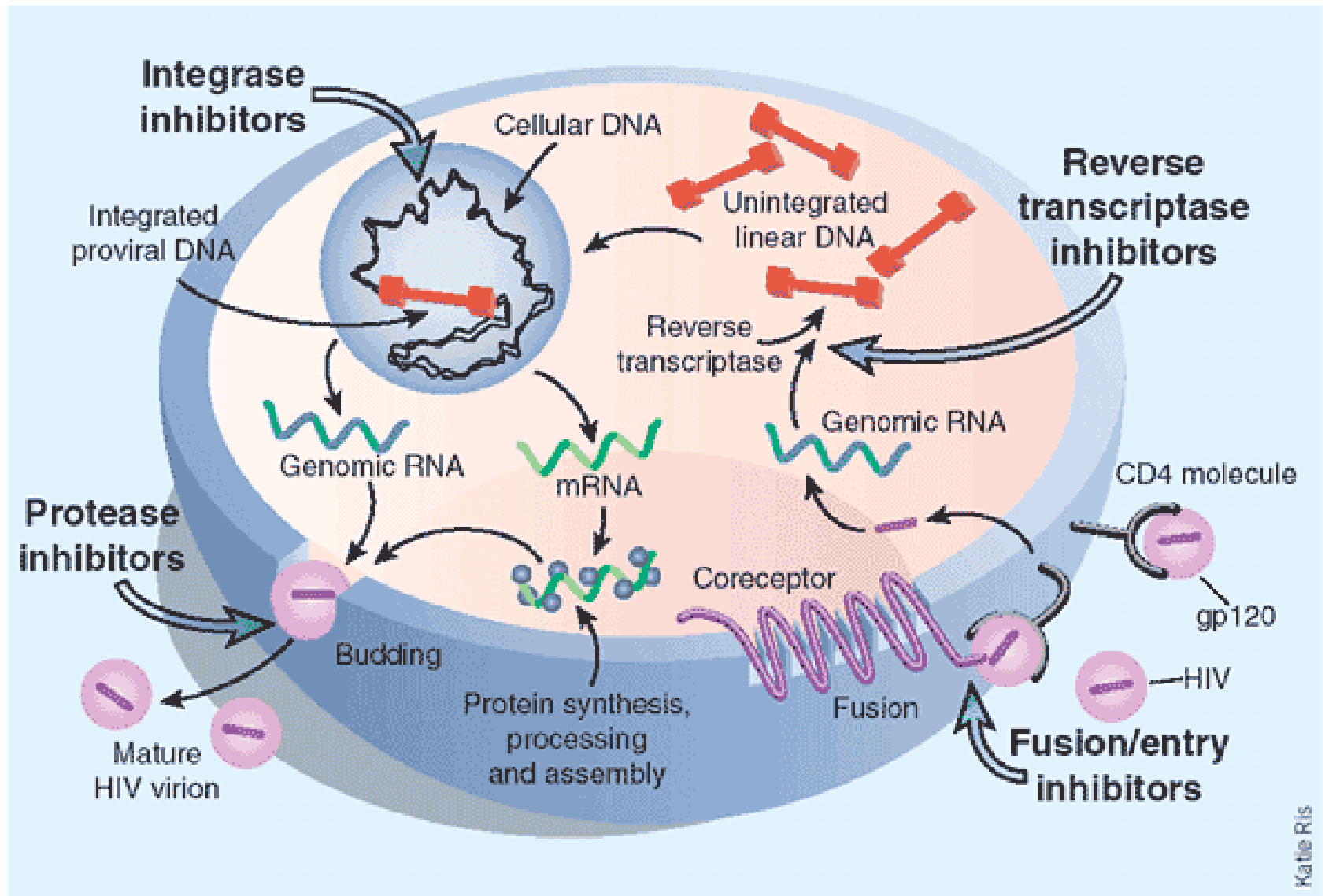
Figure 11-30 Immunobiology, 6/e. (© Garland Science 2005)

WHY IS HIV UNLIKE ANY OTHER CHRONIC INFECTION?

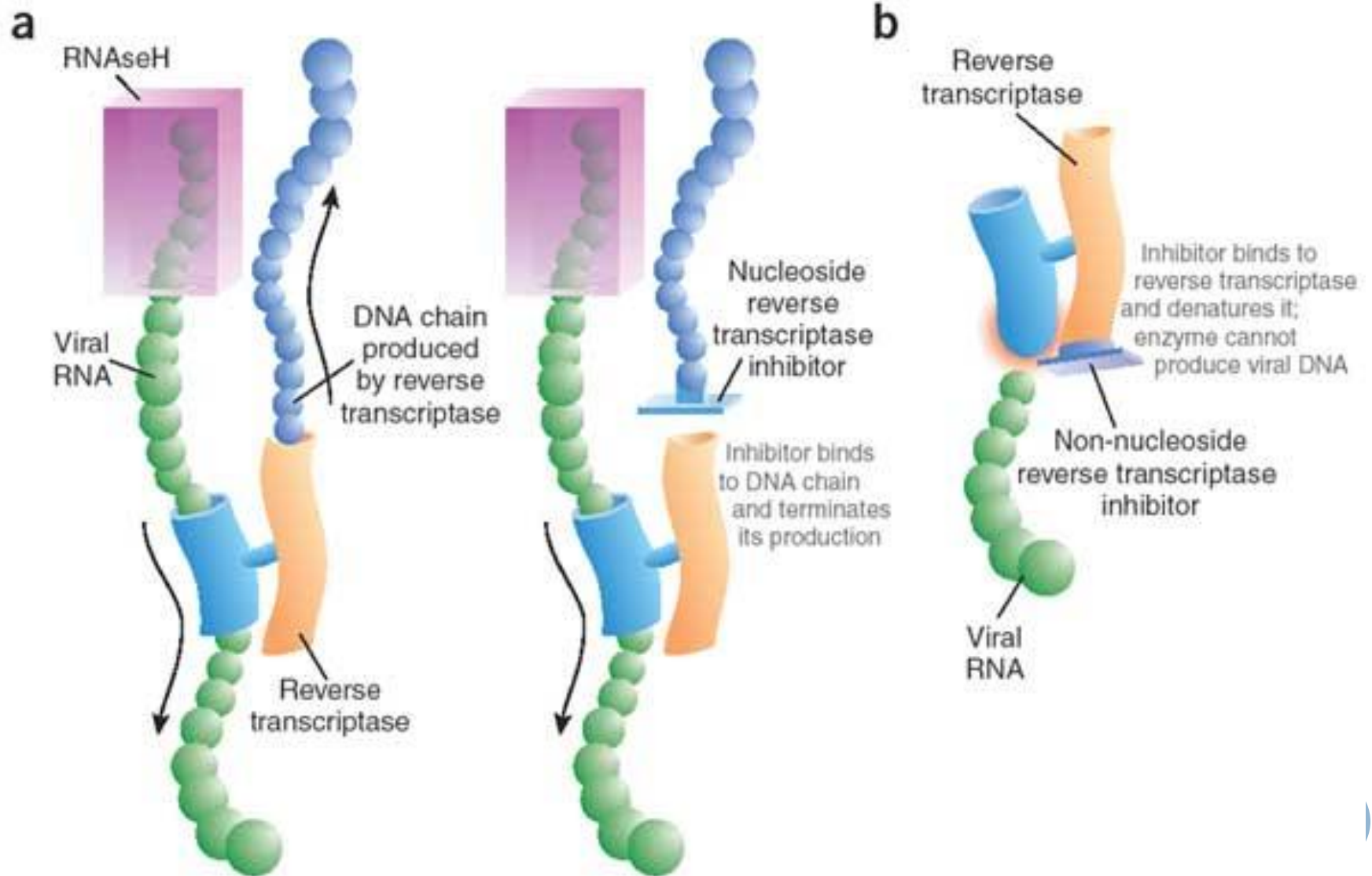
- A combination of “traditional” immune evasion mechanisms (CTL escape, antigen masking) and non-traditional (attacking immune function and cell compartments directly)



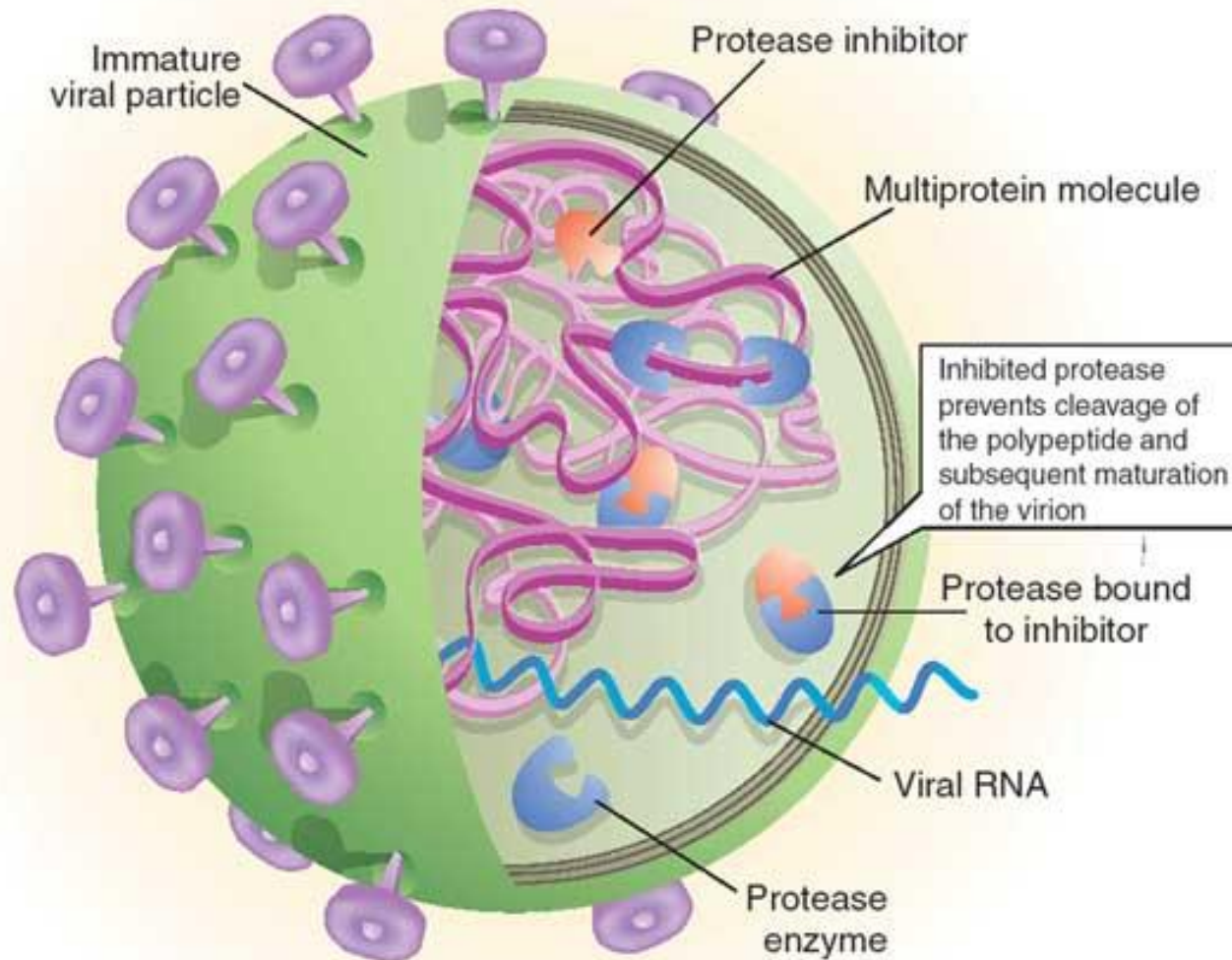
CAN INFECTION BE EFFECTIVELY CONTROLLED?



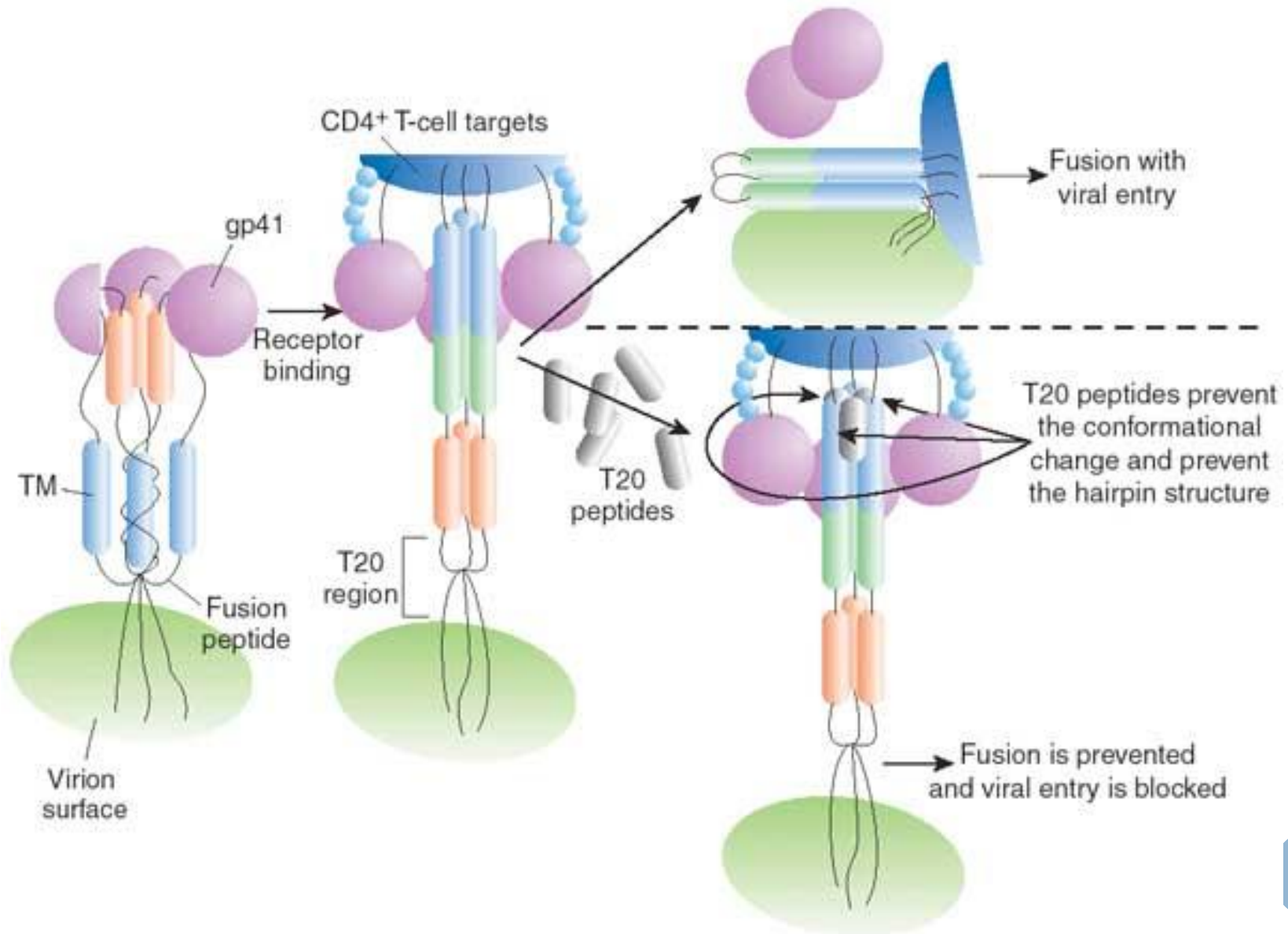
MECHANISMS OF RT INHIBITORS



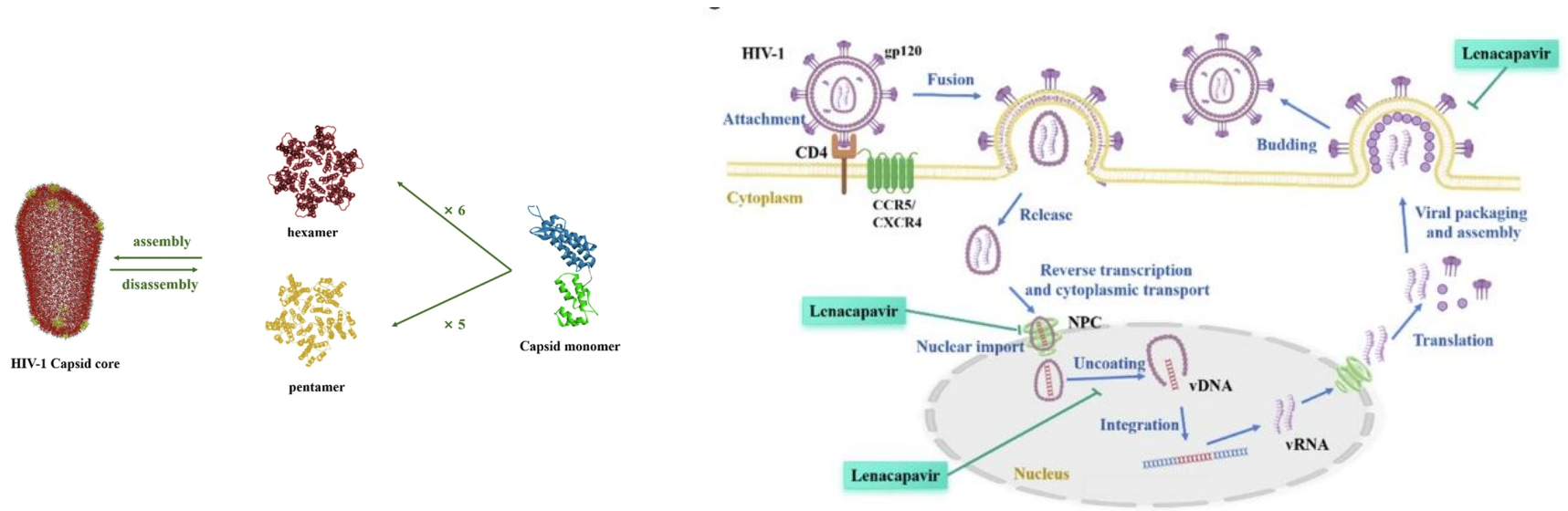
MECHANISM OF PROTEASE INHIBITORS



FUSION INHIBITORS



LENACAPAVIR FOR PRE-EXPOSURE PROPHYLAXIS

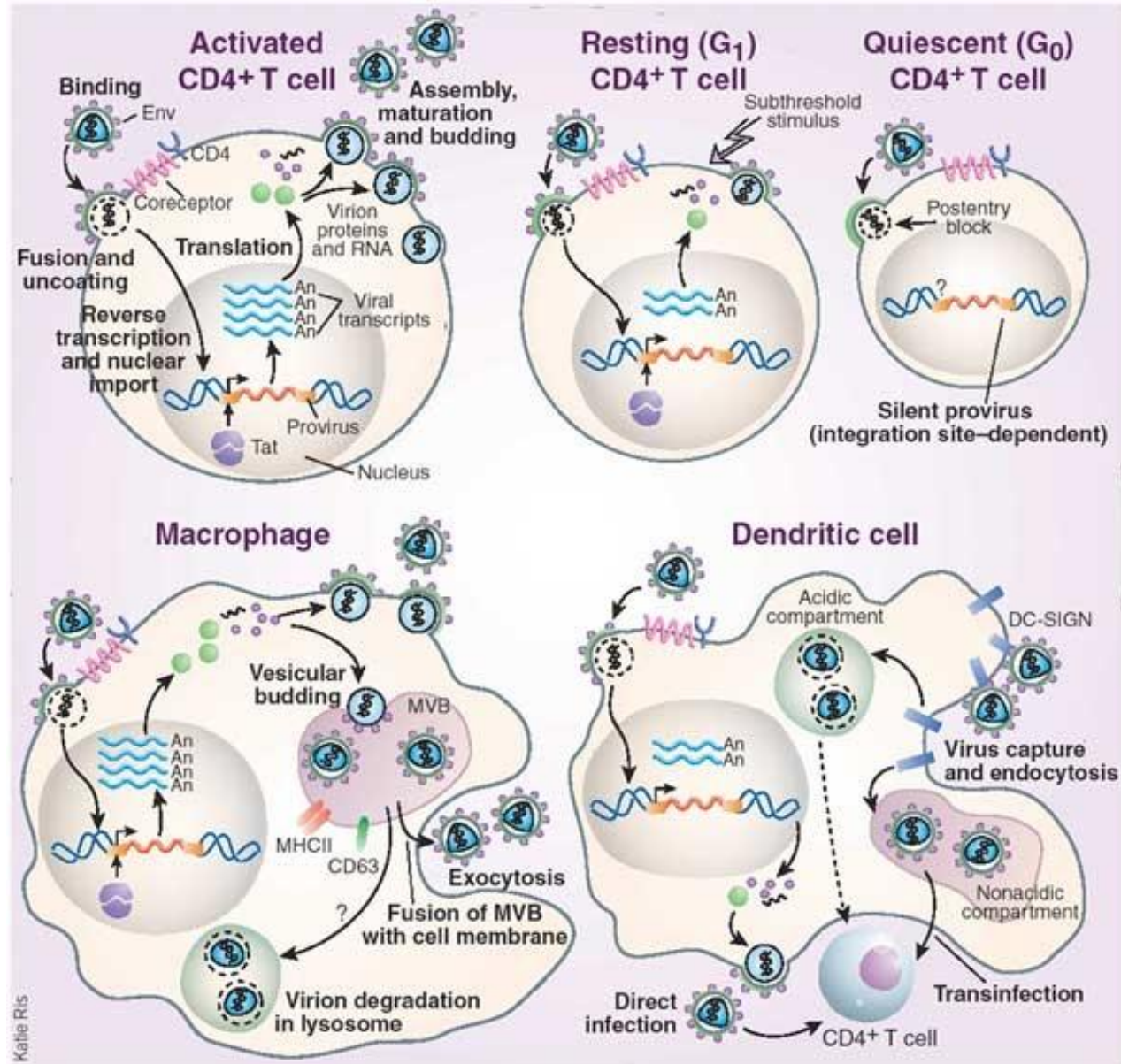


- In the mature HIV-1 capsid, this lattice is variably curved and comprises approximately 200–250 capsid hexamers along with precisely 12 capsid pentamers

LATENT RESERVOIRS OF VIRUS

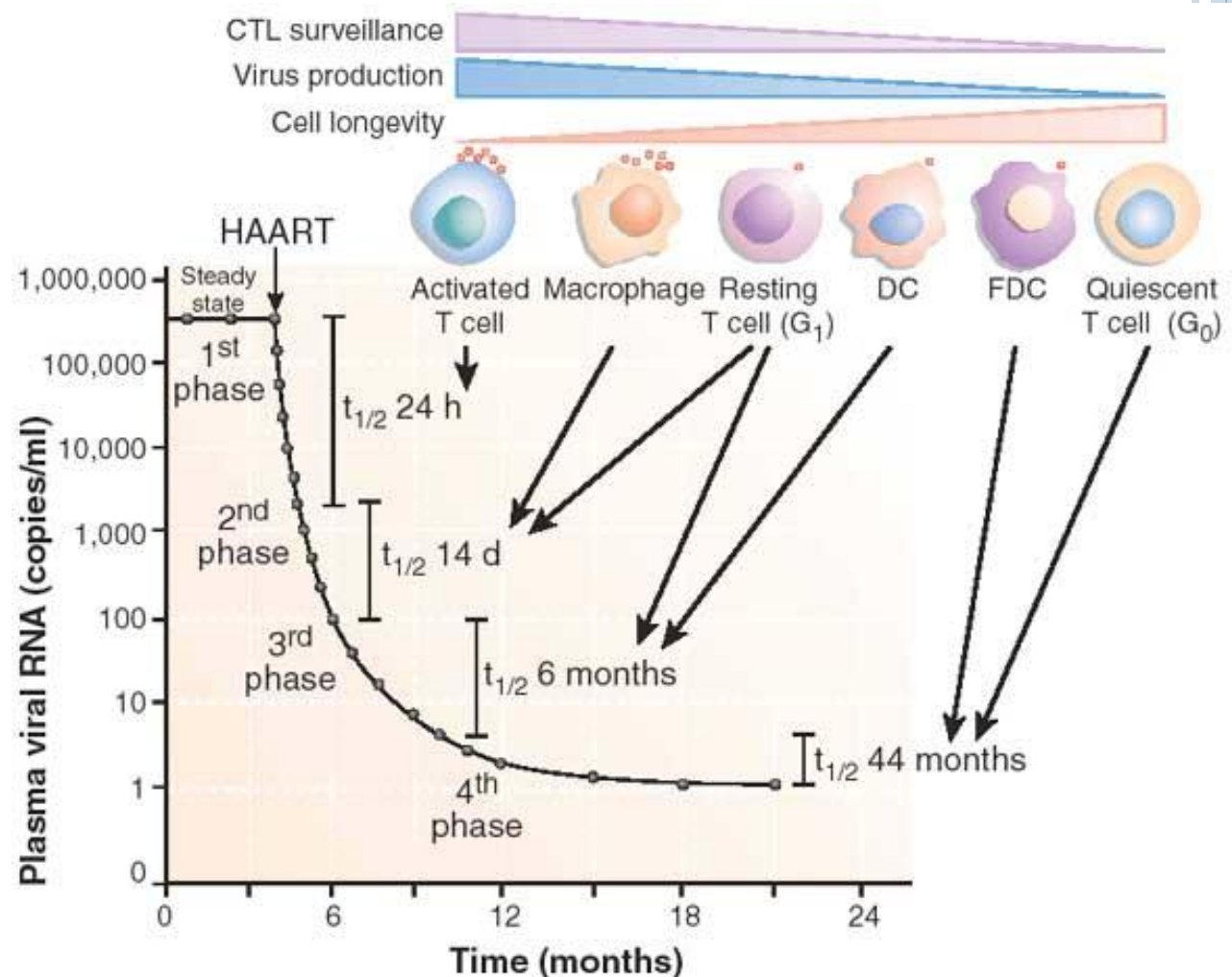
Multiple cell types can serve as latent reservoirs

“Quiescence” of infected cells constrains the possibility total viral elimination

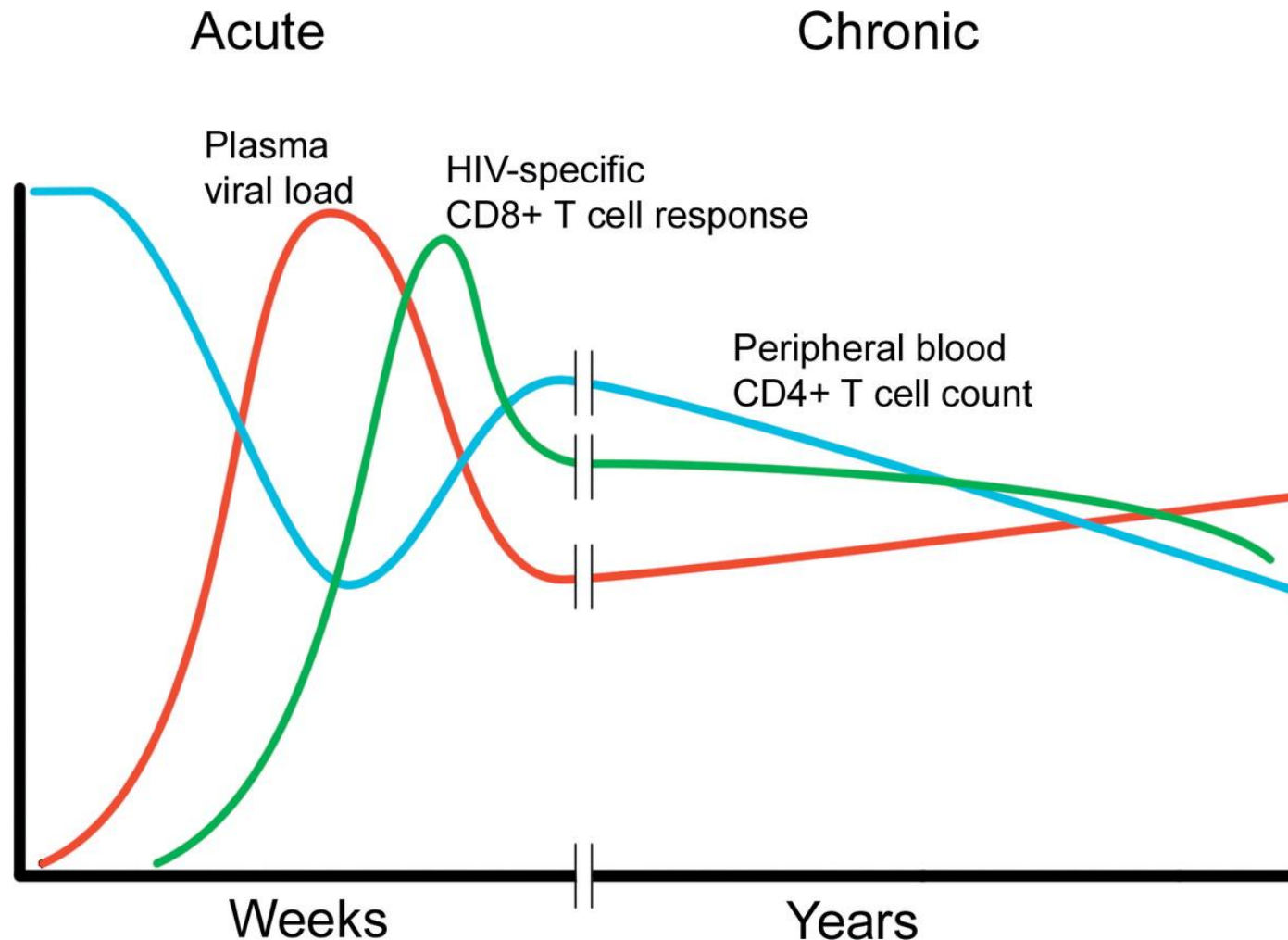


CONTRIBUTION OF INDIVIDUAL RESERVOIRS

- Steady-state virus levels result from the relative contributions and turnover of each reservoir compartment
- After viral inhibition by HAART, plasma viral RNA decays in four distinct phases allowing a dissection of each reservoir's individual contribution



CAN THE IMMUNE SYSTEM BE USED TO PREVENT OR CLEAR INFECTION?



SUMMARY OF VACCINE TRIALS IN 2006

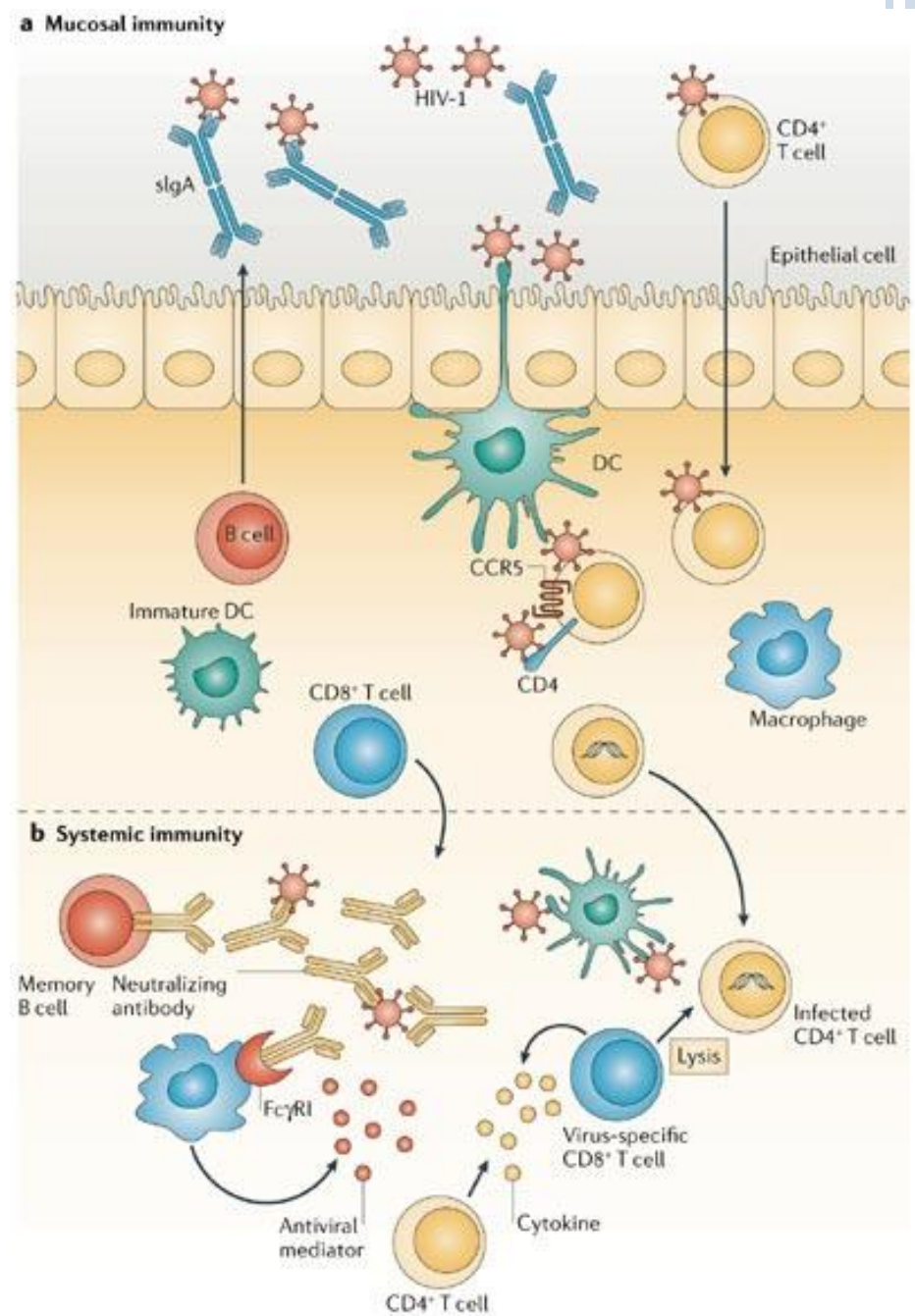
Vaccine candidate	Antigen (HIV-1 clade)	Manufacturer	Trial start date	Question being addressed
Prime with canarypox vector expressing HIV-1 genes	env (B, E), gag/pol (B)	Sanofi-Pasteur	October 2003	Will a gp120 protein vaccine that did not confer protection when used alone be useful in combination with a live, recombinant pox vector prime?
Boost with gp120 protein	gp120 (B, E)	Vaxgen		
Replication-defective adenovirus serotype 5 expressing HIV-1 genes	gag, pol, nef (B)	Merck	December 2004	Will an adenovirus-based vector vaccine confer a clinical benefit in individuals who become infected after vaccination?
Prime with plasmid DNA encoding HIV-1 genes	gag, pol, nef (B), env (A, B, C)	Vical, VRC	September 2005	Will a prime–boost strategy using DNA- and adenovirus-based vaccines encoding envelope proteins from three HIV-1 clades, as well as viral structural proteins, confer a benefit?
Boost with replication-defective adenovirus serotype 5 expressing HIV-1 genes	gag, pol (B), env (A, B, C)	GenVec, VRC		

Further information on [ongoing trials of preventative AIDS vaccines](#) can be found in the 2006 International AIDS Vaccine Initiative report. env, envelope; gag, group-specific antigen; gp120, glycoprotein 120; nef, negative factor; pol, polymerase; VRC, Vaccine Research Center, National Institutes of Health, Maryland, USA.

Letvin *Nature Reviews Immunology* **6**, 930–939 (December 2006) | doi:10.1038/nri1959

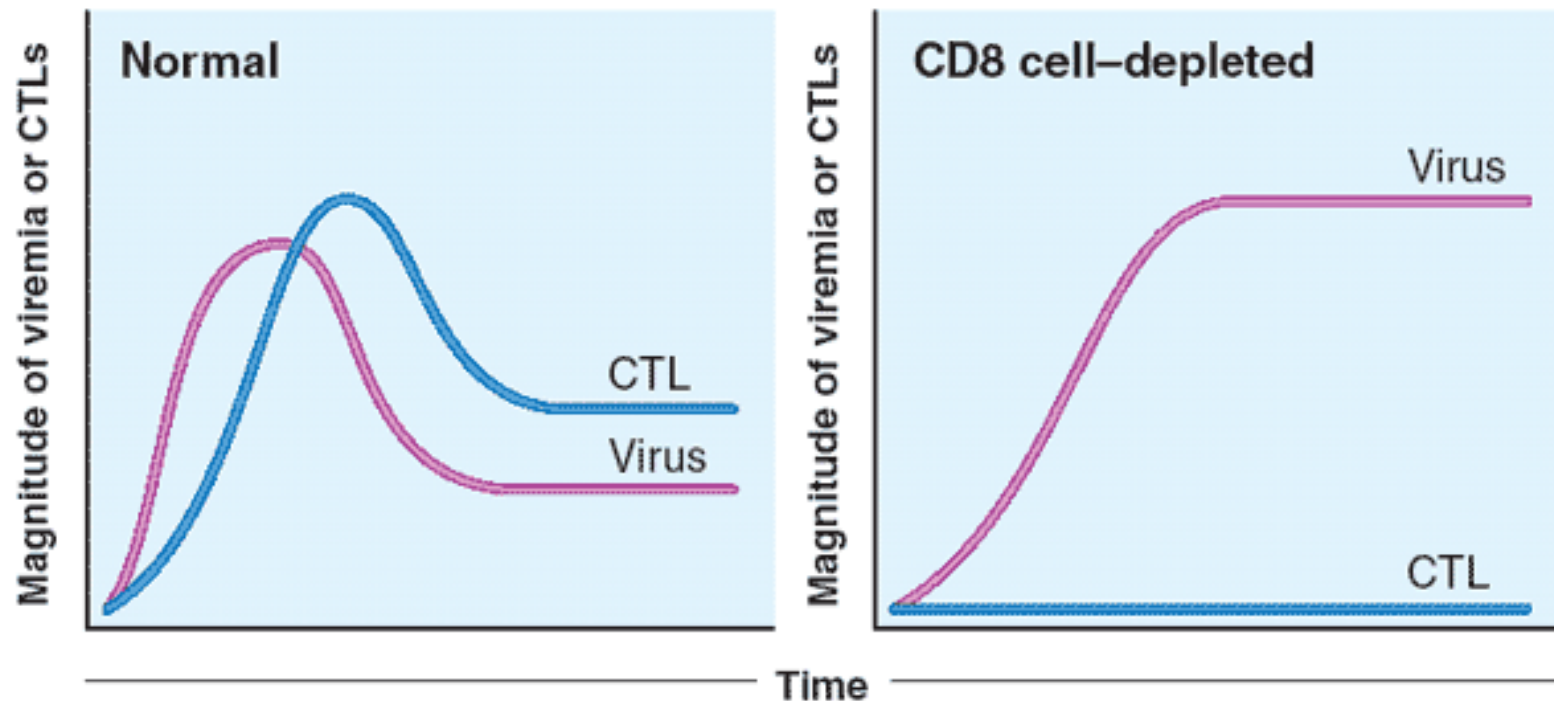
MECHANISMS OF IMMUNE PROTECTION

- “Standard” immunological protection mechanisms, including antibody, clearance by phagocytic cells and Fc receptors, and cytotoxic killing of infected cells all function to limit infection and control long-term viral loads
- The loss of effective immune control is what leads to the development of AIDS, therefore the immune response in principle is an effective tool for viral control and clearance



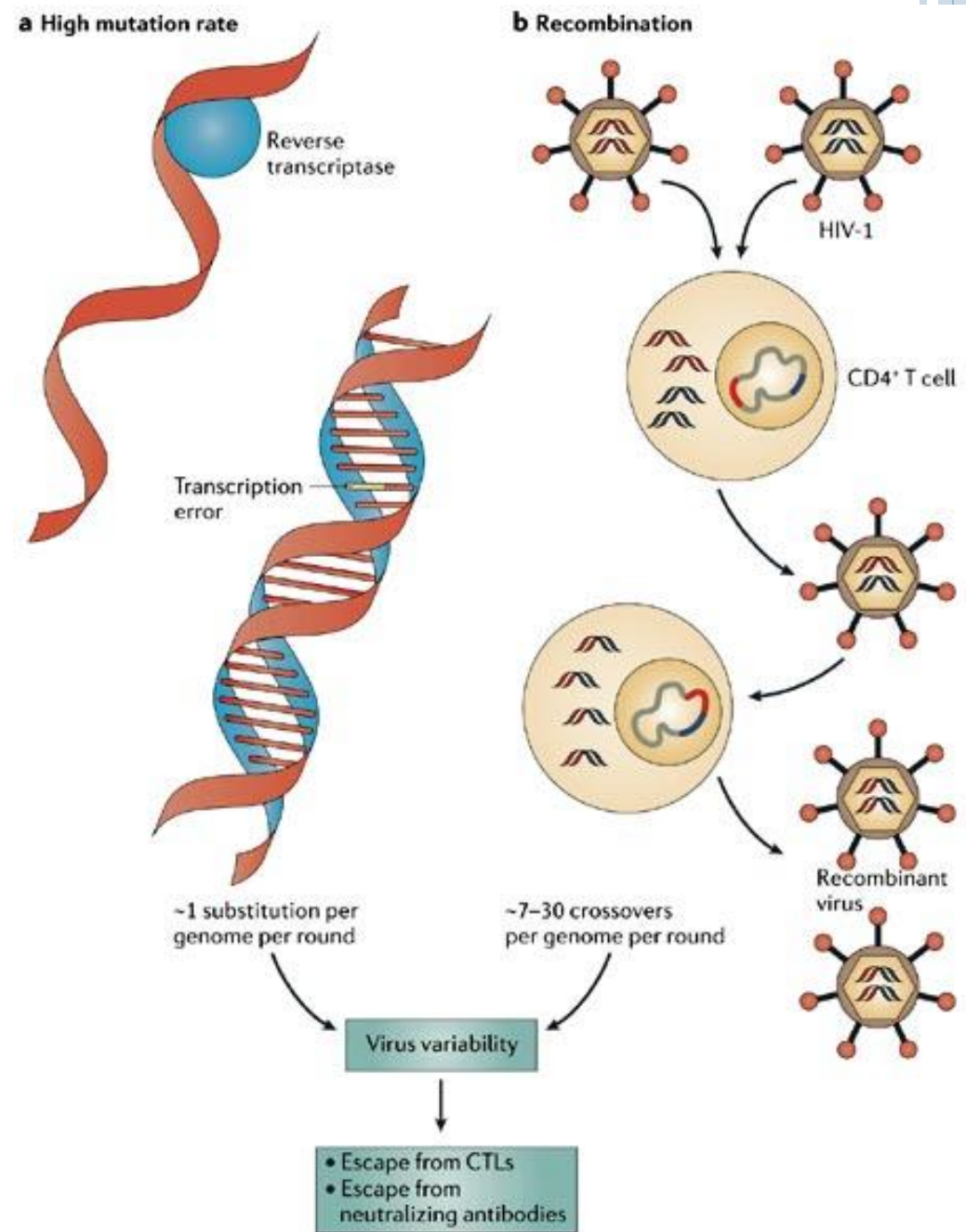
CD8 T CELLS PROVIDE SIGNIFICANT VIRAL CONTROL DURING THE CHRONIC PHASE OF INFECTION

- CD8 depletion in SIV-infected animals leads to rapid increase in viral titers and pathogenesis of disease



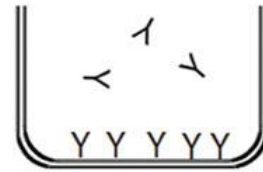
VIRAL IMMUNE ESCAPE MECHANISMS

- “Antigenic drift” from the very high rate of mutation of the RT enzyme allows rapid escape from individual antibody and CTL responses
- Epitopes are constrained by structural/functional requirements



HOW DO WE ASSAY FOR T CELL RESPONSES IN HIV INFECTED INDIVIDUALS?

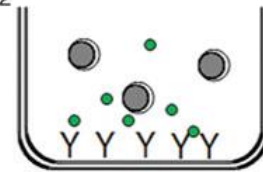
Step 1



Coat nitrocellulose plate with coating IFN- γ antibody overnight.

Wash plate six times with sterile PBS containing 1% FCS for blocking.

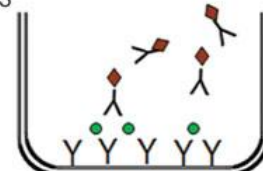
Step 2



Incubate cytokine-secreting cells with respective stimulus (e.g., peptide, PHA, SEB) overnight at 37 °C 5% CO₂.

Wash plate six times with sterile PBS.

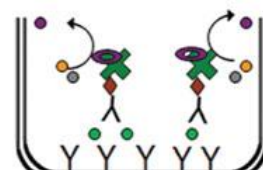
Step 3



Incubate with biotinylated detection IFN- γ antibody for 1.5 h at room temperature.

Wash plate six times with sterile PBS.

Step 4



Incubate with alkaline phosphatase-linked streptavidin for 1 h in the dark.

Wash plate six times with sterile PBS.

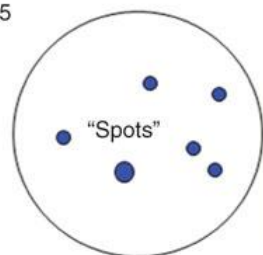
Add color reagents for 10–20 min.

Decontaminate plate by adding 0.05% Tween-20.

Wash color reagents off.

Dry plate and count spots.

Step 5



Legend

Y IFN- γ capture antibody

• IFN- γ

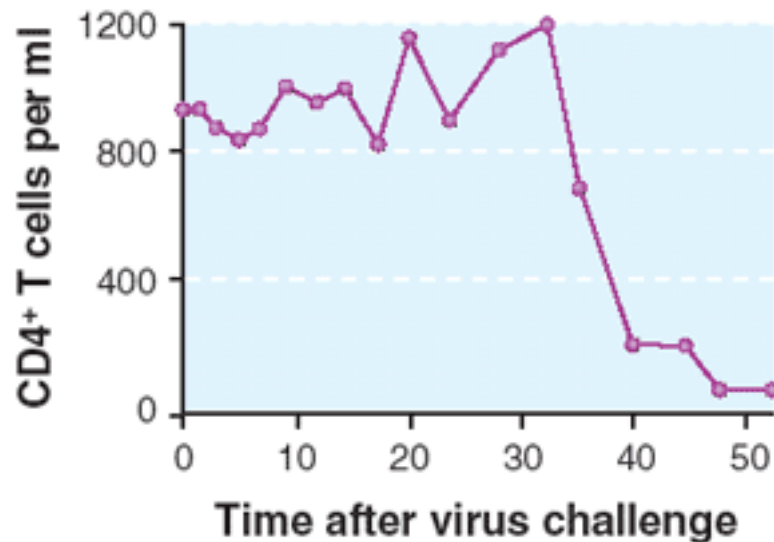
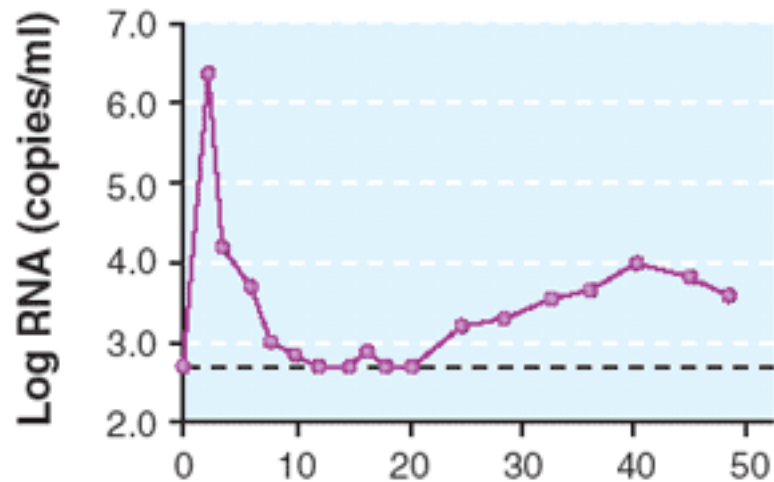
● Cytokine-secreting cells

◆ Biotinylated detection IFN- γ antibody

⊕ Alkaline phosphatase-linked streptavidin

● Color reagent

IMMUNODOMINANT EPITOPE ESCAPE CAN LEAD TO LOSS OF VIRAL CONTROL



Gag p11C (181–189) sequences

C T P Y D I N Q M

Week 0 - - - - - (15/15)

Week 14 - - - - - (8/8)

Week 20 - I - - - - - (10/10)

Week 24 - I - - - - - (11/11)

Week 28 - I - - - - - (11/11)

Week 36 - I - - - - - (11/11)

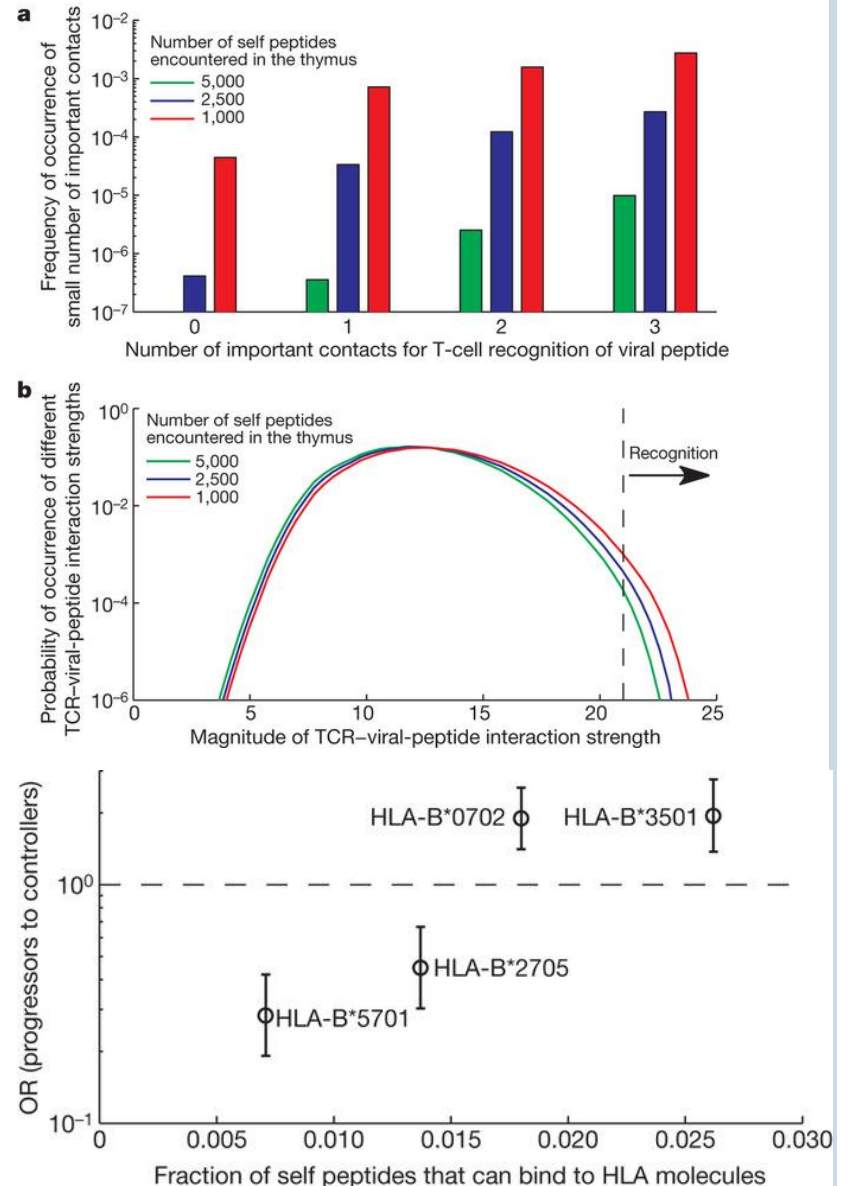
EEK 44 - I - - - - - (10/10)

RECENT REPORTS RELATING MHC HAPLOTYPE TO HIV CONTROL

Nature 465, 350–354 (20 May 2010) Effects of thymic selection of the T-cell repertoire on HLA class I-associated control of HIV infection

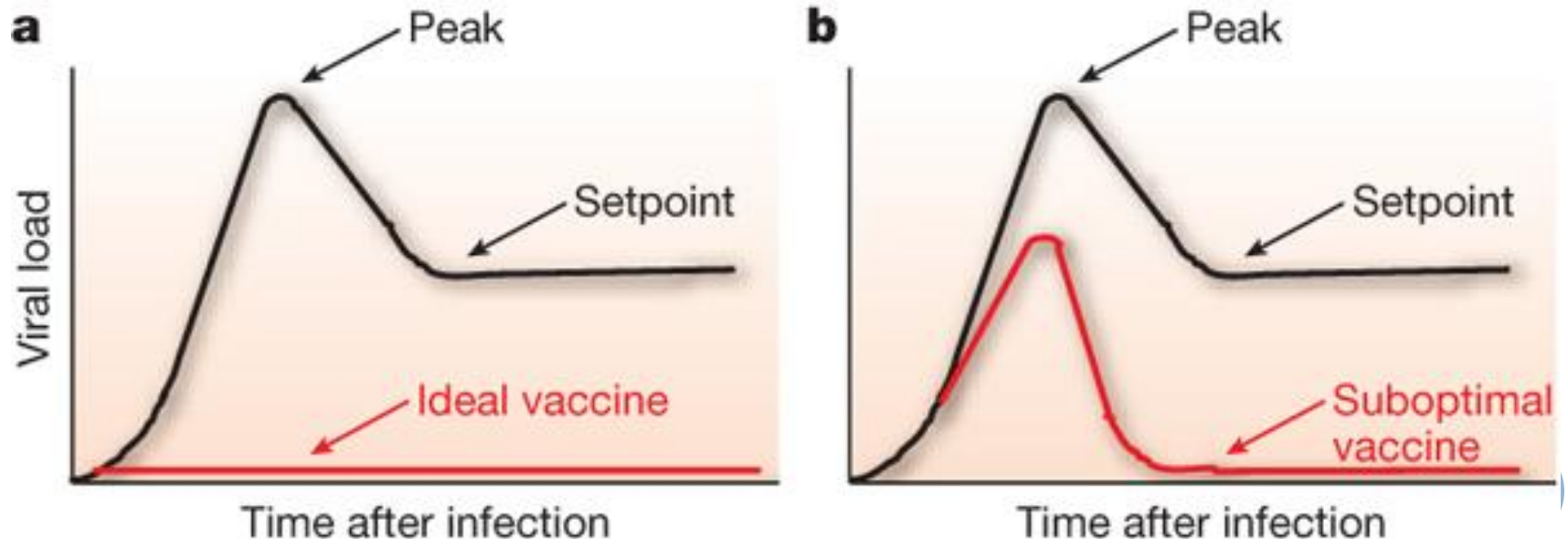
Andrej Košmrlj, Elizabeth L. Read, Ying Qi, Todd M. Allen, Marcus Altfeld, Steven G. Deeks, Florencia Pereyra, Mary Carrington, Bruce D. Walker & Arup K. Chakraborty

- Relating the breadth of the TCR repertoire (how many different T cell receptors does the body make?) to the MHC haplotype (the more self peptides available for negative selection, the narrower (and less “cross-reactive” the TCR repertoire)
- Less cross-reactive TCR repertoires are then associated with poor control



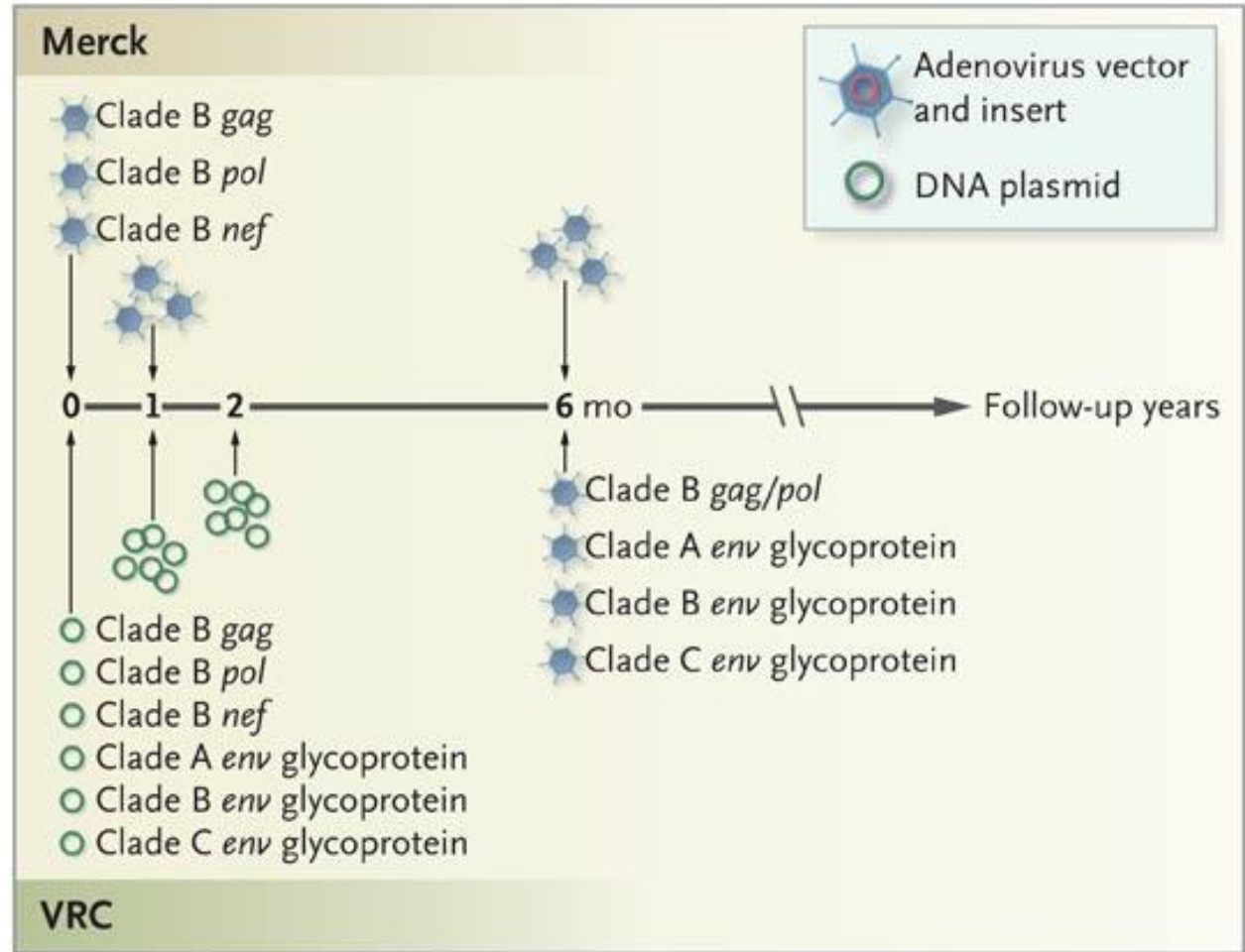
VACCINE EXPECTATIONS

- Since viral load “set point” is a key predictor of disease progression and pathogenesis, even a suboptimal vaccine could be of use in highly endemic areas to protect against disease and spread (we’ll talk more about this when we get to malaria)



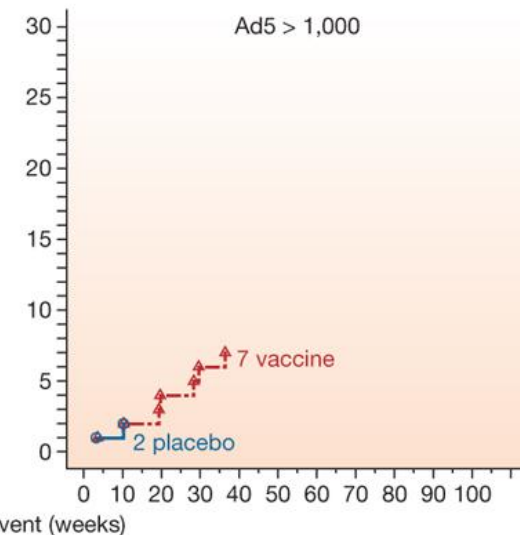
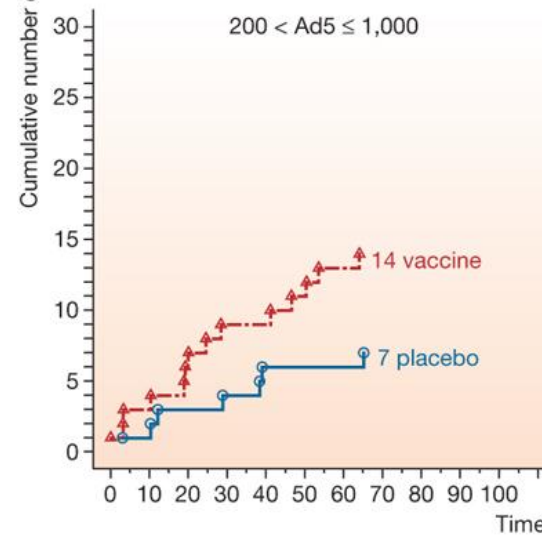
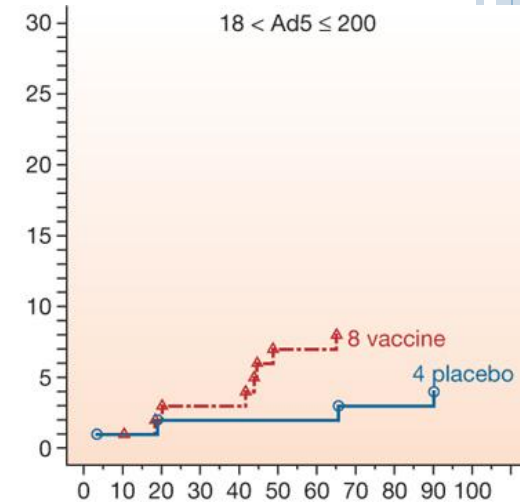
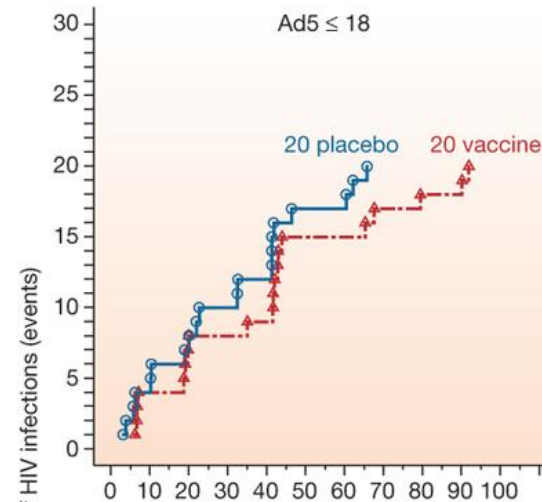
THE MERCK VACCINE

- Use of a viral vector has been shown experimentally to boost cellular responses, by delivering more antigen with the proper innate/PAMP signals



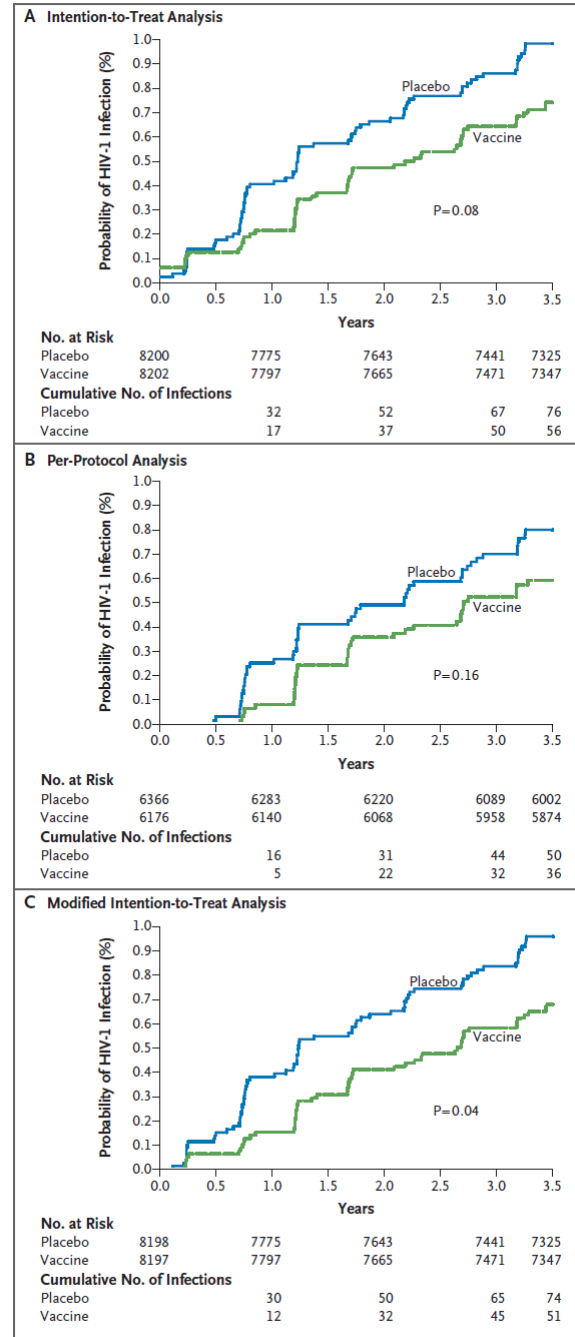
MERCK VACCINE FAILURE

- Not only did the Merck Vaccine fail to protect, there appeared to be an enhancement of infection in vaccinees who had relatively higher pre-existing antibody titers to the viral vector
- This failure led to the cancellation of other vaccine trials based on a similar approach
- HVTN-505 just halted in April 2013—also Ad5 based (41 vacc inf, 30 placebo)

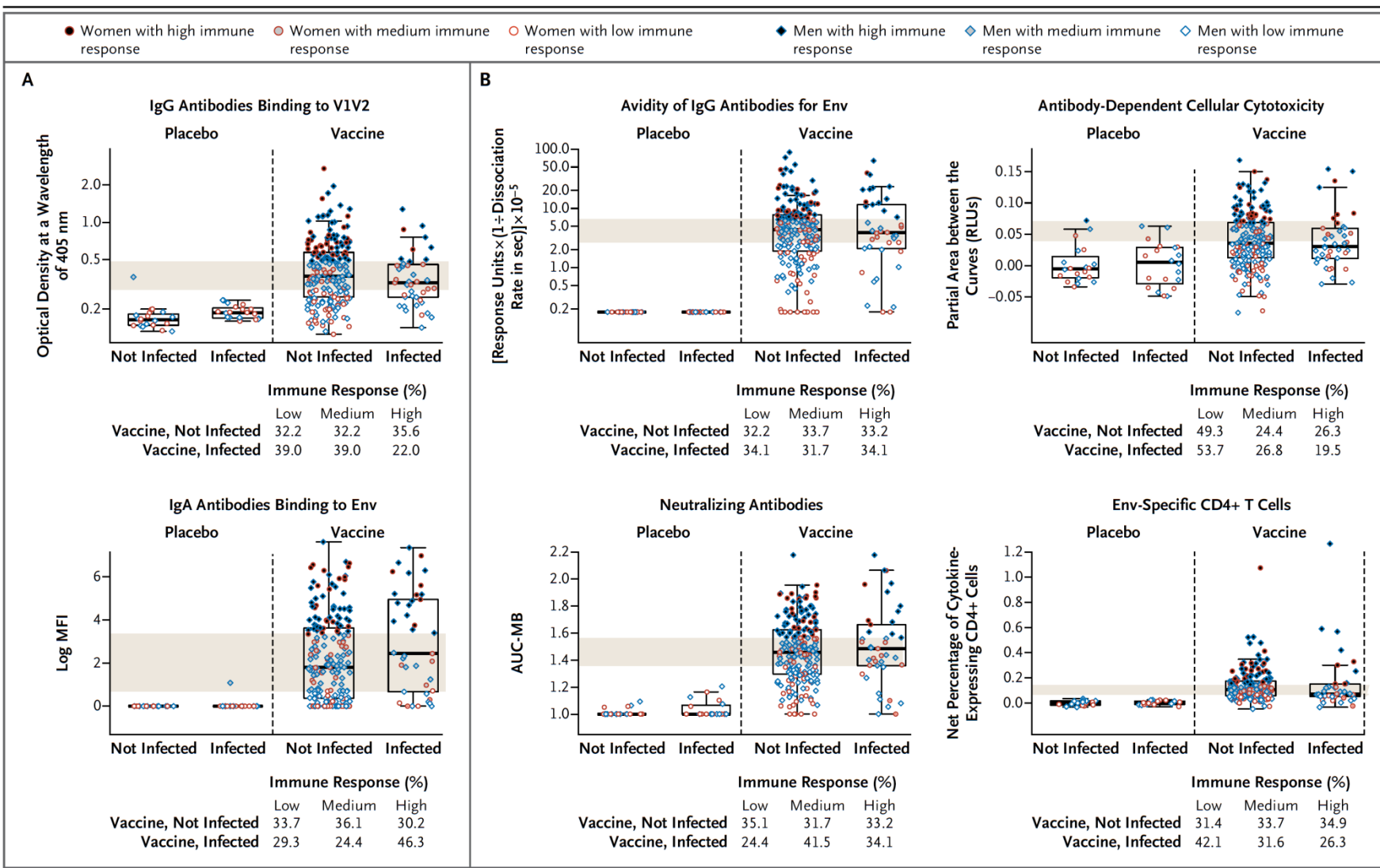


A PROTECTIVE VACCINE? RV144 TRIAL

- ALVAC/AIDSVAX Prime boost-boost vaccine (canarypox followed by protein boost, gp120 based)
- 16,402 vaccinees
- Vaccine efficacy was 31.2%
- No mitigation of viral load in those that did become infected



IMMUNE CORRELATES OF HIV RISK



POINTS FOR DISCUSSION

- HIV is a unique pathogen in that it targets the immune system directly—playing “offense”—killing or dysregulating the cells that specifically target it and “defense”, employing more conventional immune escape mechanisms
- Despite this, the immune response, both antibody and CTLs, provide an important level of control over the virus for an extended period of time, keeping the reservoir relatively stable
- Vaccines could in principle employ similar strategies, but drugs are still the most effective treatment tool

