Viral Infections and Immunology

Unit 4
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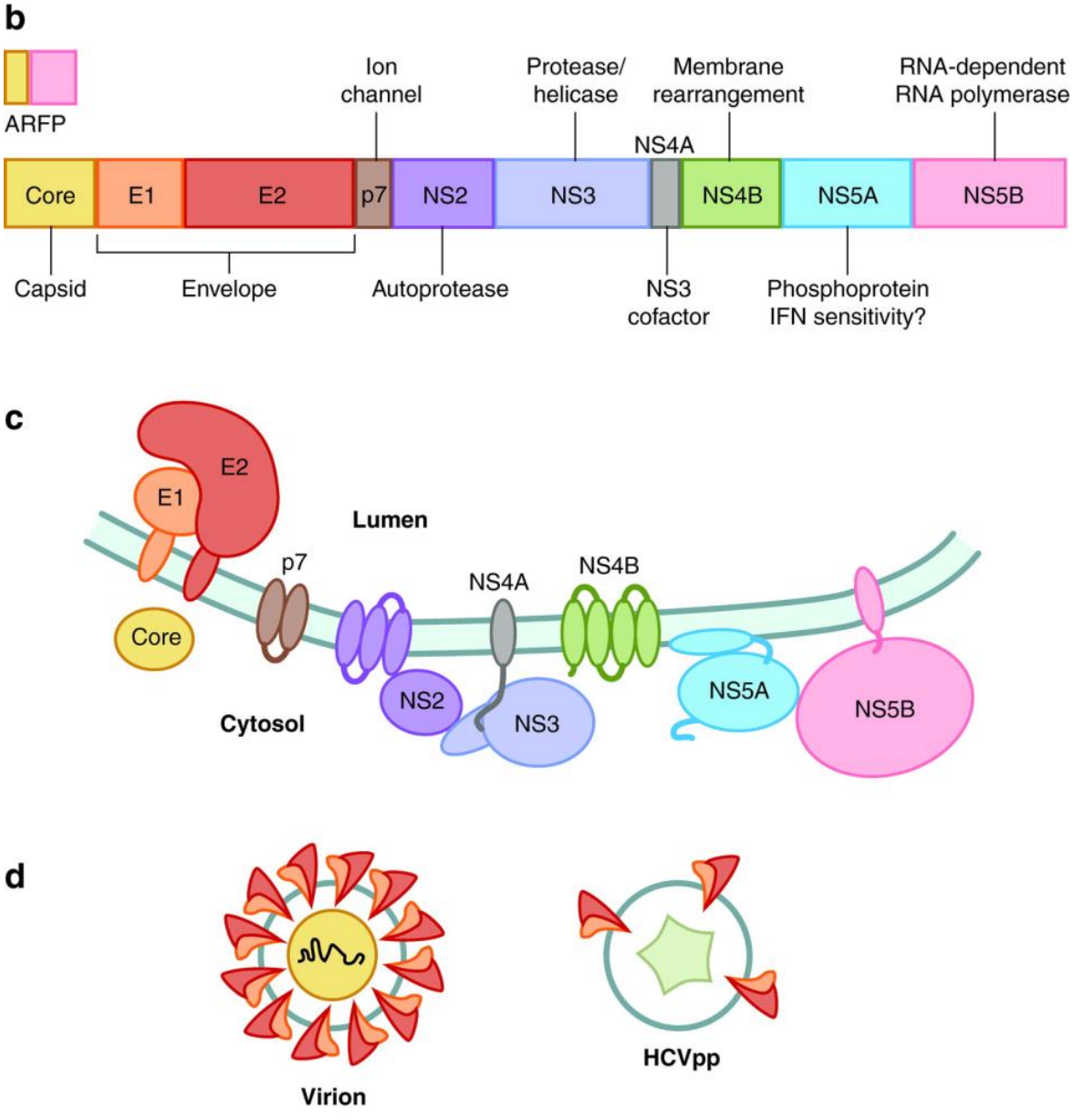
Hepatitis C virus

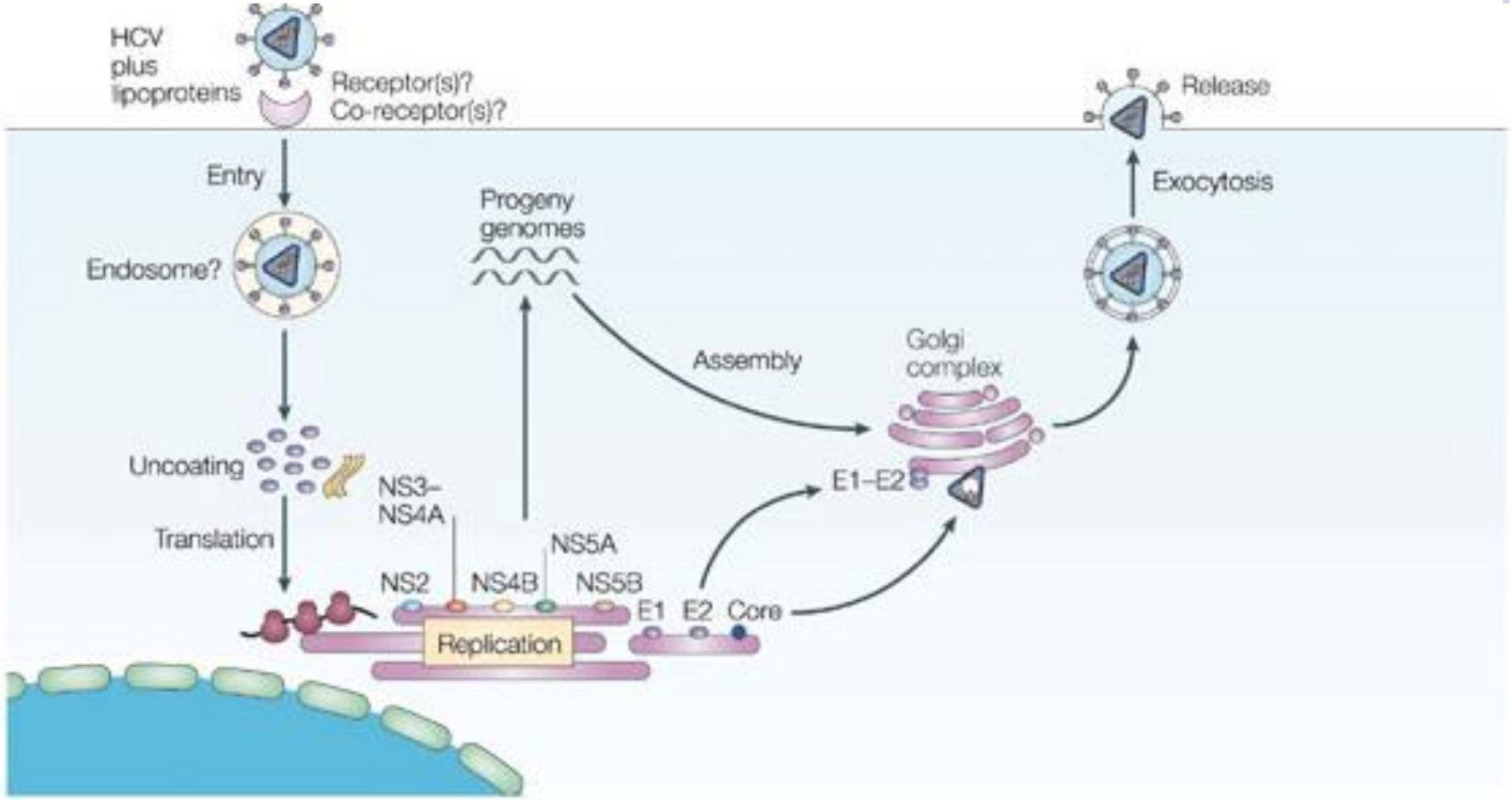
- Enveloped, positive strand RNA virus, *Flaviviridae*
- Isolated in 1989, treatments first emerged in early 1990s
- ~120 million-200 million infections worldwide, number one indication for liver transplant in the U.S.
- 10^{12} viral particles produced/day, ½ life 3 hours in circulation
- Six major 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



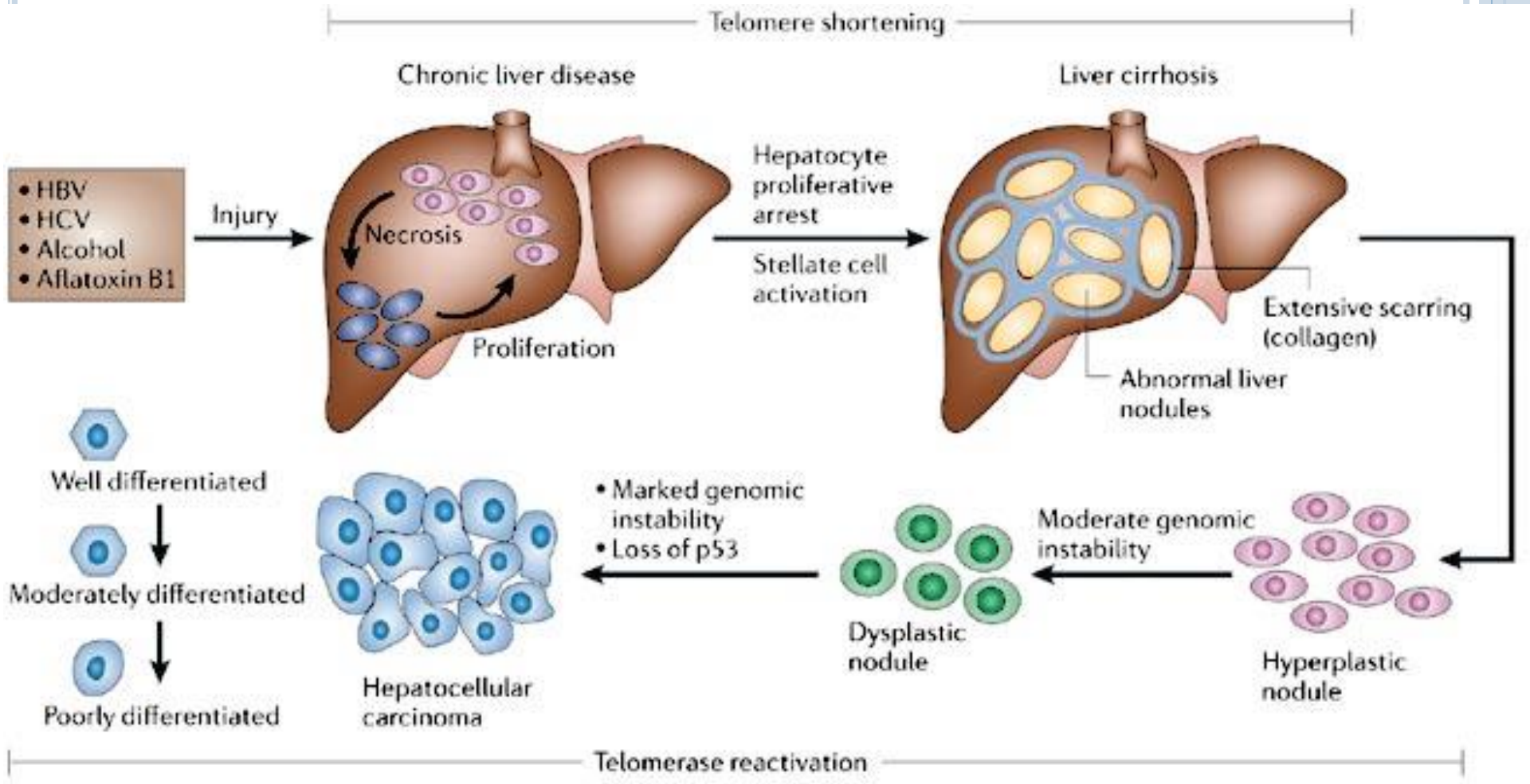
Dustin LB, Rice CM. 2007.
Annu. Rev. Immunol. 25:71–99

HCV LIFE CYCLE

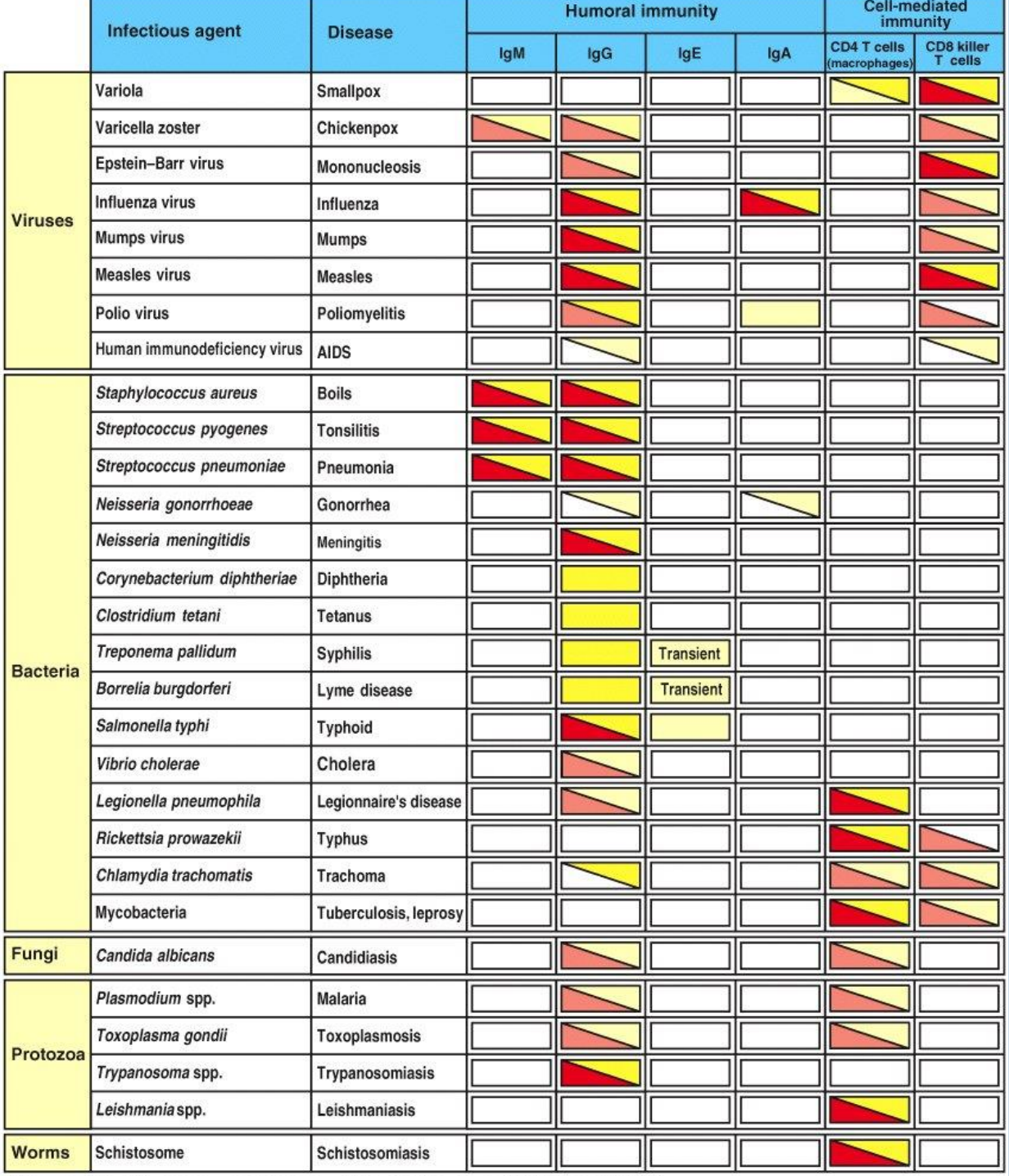


HCV Life Cycle 2

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



Specific Clearance Mechanisms For Pathogen Classes (keep in mind redundancy)



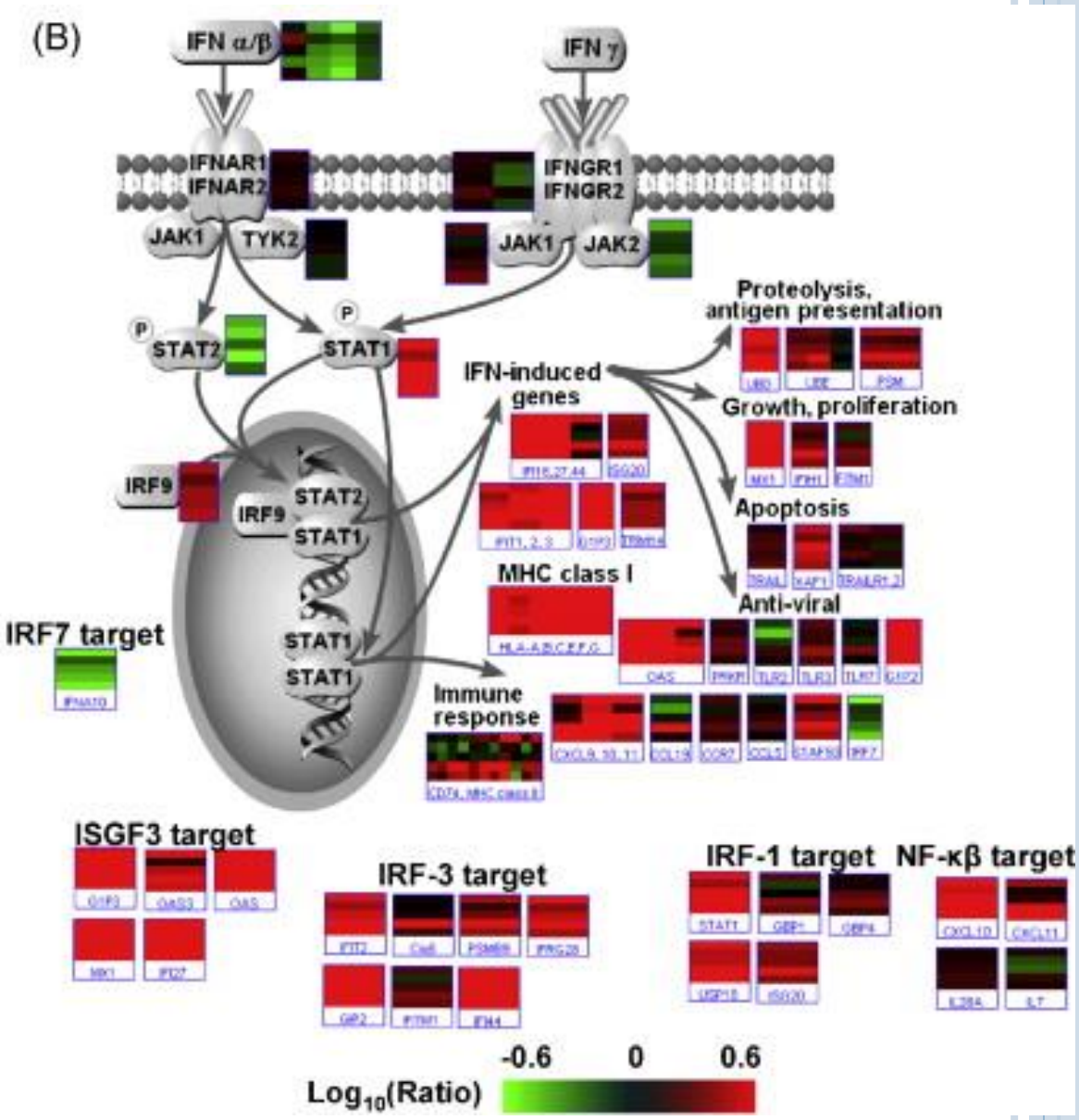
	Infectious agent	Disease	Humoral immunity				Cell-mediated immunity	
			IgM	IgG	IgE	IgA	CD4 T cells (macrophages)	CD8 killer T cells
Viruses	Variola	Smallpox						
	Varicella zoster	Chickenpox						
	Epstein–Barr virus	Mononucleosis						
	Influenza virus	Influenza						
	Mumps virus	Mumps						
	Measles virus	Measles						
	Polio virus	Poliomyelitis						
	Human immunodeficiency virus	AIDS						
Bacteria	*Staphylococcus aureus*	Boils						
	Streptococcus pyogenes	Tonsilitis						
	Streptococcus pneumoniae	Pneumonia						
	Neisseria gonorrhoeae	Gonorrhea						
	Neisseria meningitidis	Meningitis						
	Corynebacterium diphtheriae	Diphtheria						
	Clostridium tetani	Tetanus						
	Treponema pallidum	Syphilis			Transient			
	Borrelia burgdorferi	Lyme disease			Transient			
	Salmonella typhi	Typhoid						
	Vibrio cholerae	Cholera						
	Legionella pneumophila	Legionnaire's disease						
	Rickettsia prowazekii	Typhus						
	Chlamydia trachomatis	Trachoma						
	Mycobacteria	Tuberculosis, leprosy						
Fungi	*Candida albicans*	Candidiasis						
Protozoa	*Plasmodium* spp.	Malaria						
	Toxoplasma gondii	Toxoplasmosis						
	Trypanosoma spp.	Trypanosomiasis						
	Leishmania spp.	Leishmaniasis						
Worms	Schistosome	Schistosomiasis						

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

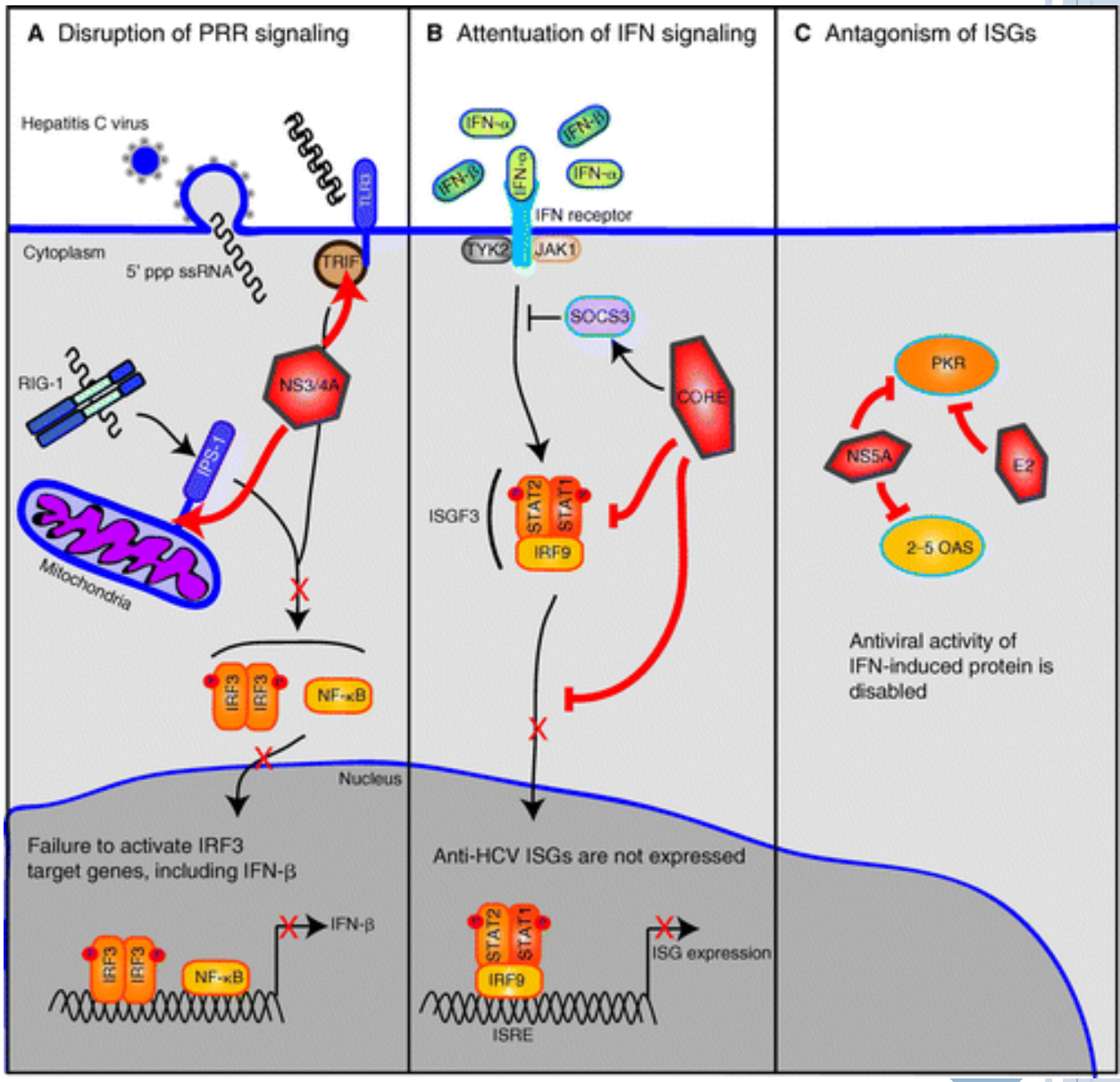
Induction of innate Immunity in Patients

- 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 resposnes
  - MHC I expression
  - Chemokine secretion and recruitment of responder cells



Innate Recognition of HCV

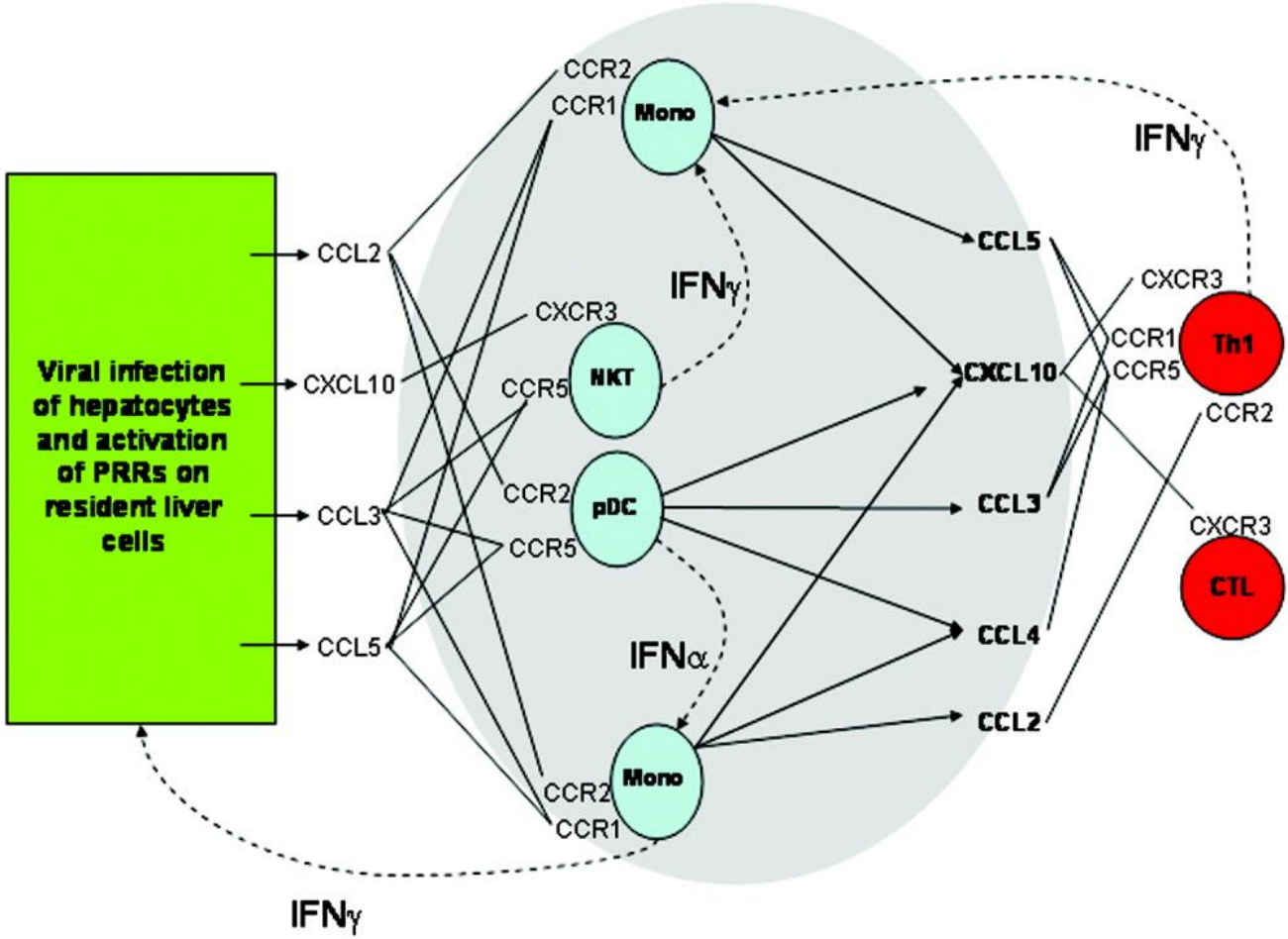
- The generation of dsRNA structures in HCV replication leads to recognition by multiple innate pathways
- HCV subverts these pathways by sequestering or cleaving key components of innate recognition
- The effects are both qualitative and quantitative on the ensuing innate response



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

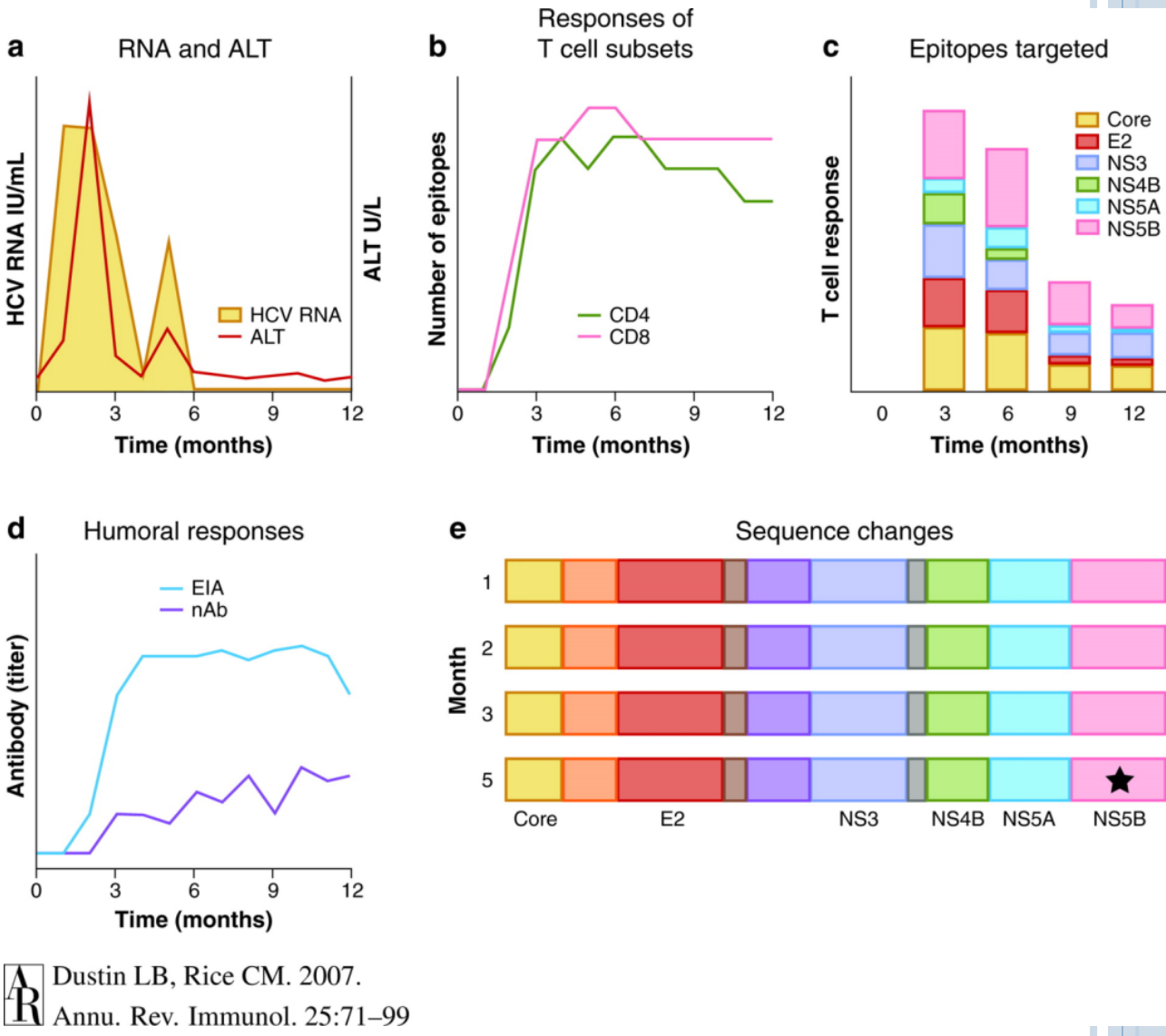
Innate activation of adaptive responses

- 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 CD_4 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 CD_4 and CD8 cells (the role of antibody is controversial)



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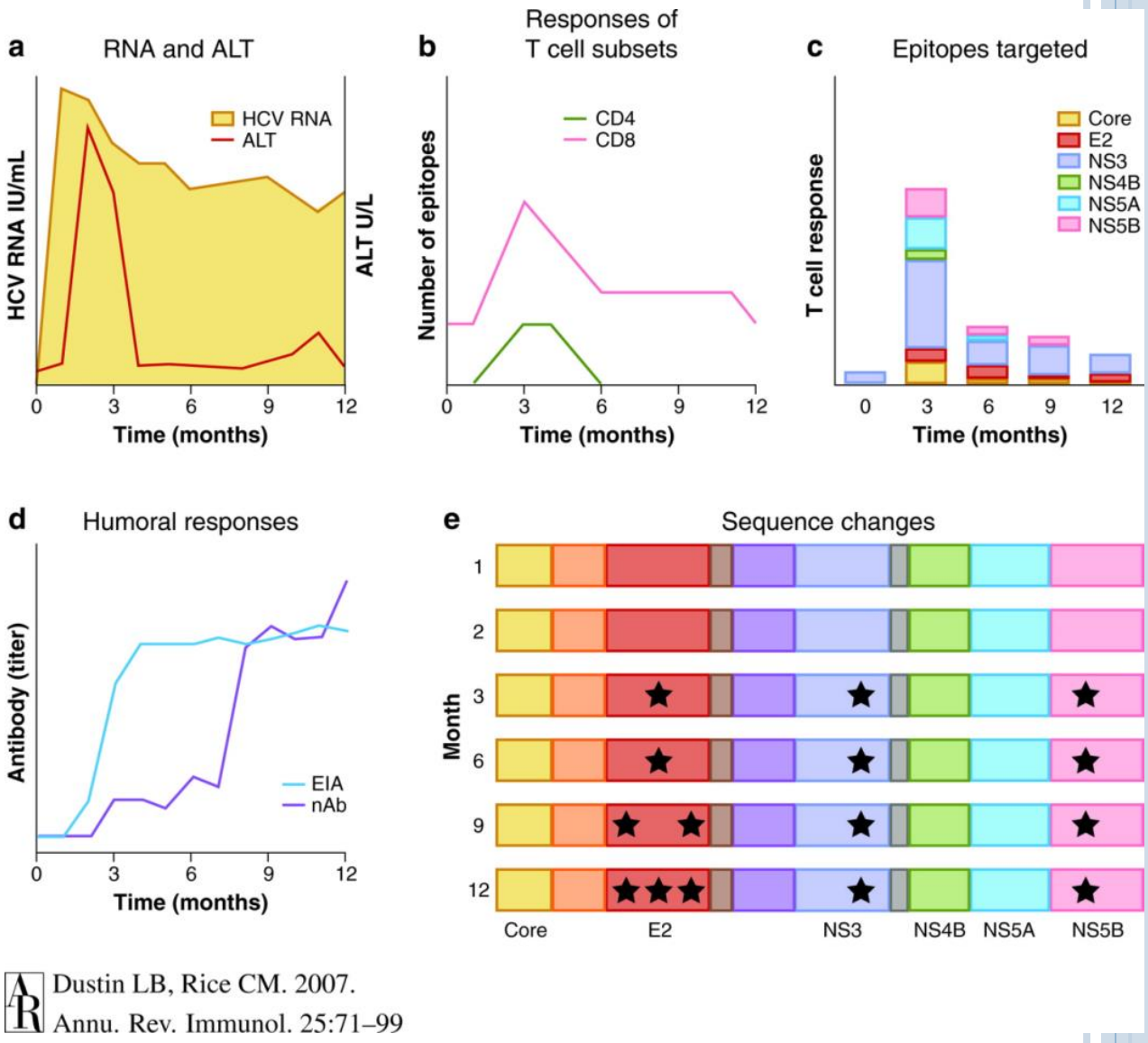
Chronic HCV infections result from poor T cell control, epitope escape and Limited Repertoires

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



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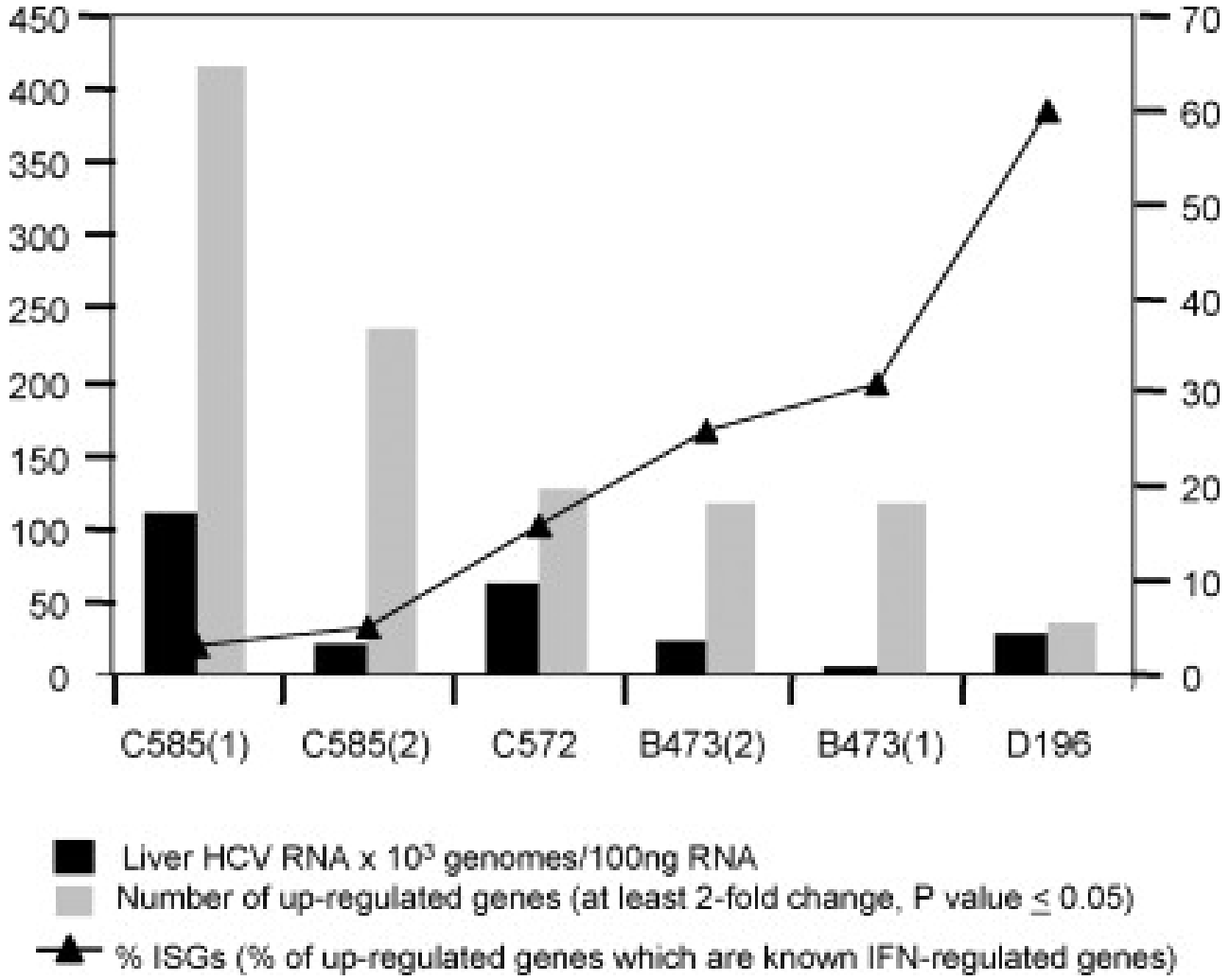
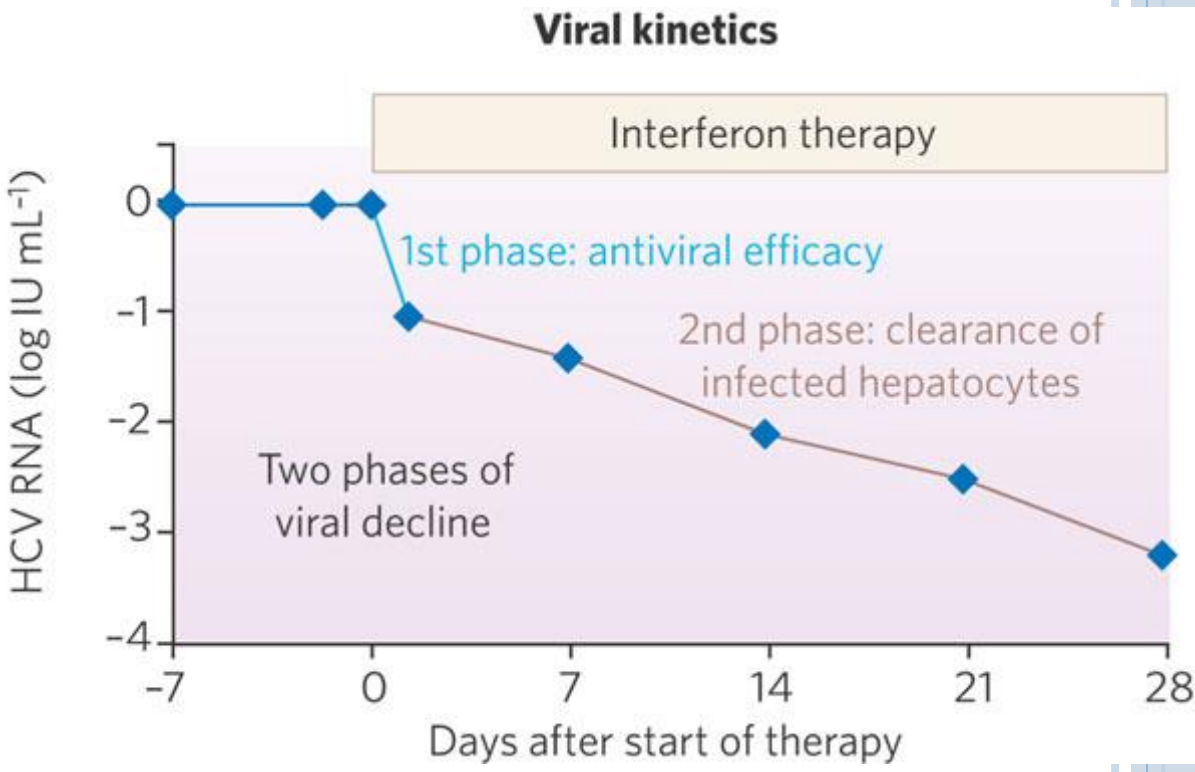
Control of Acute infection correlates with Interferon-Induced Genes



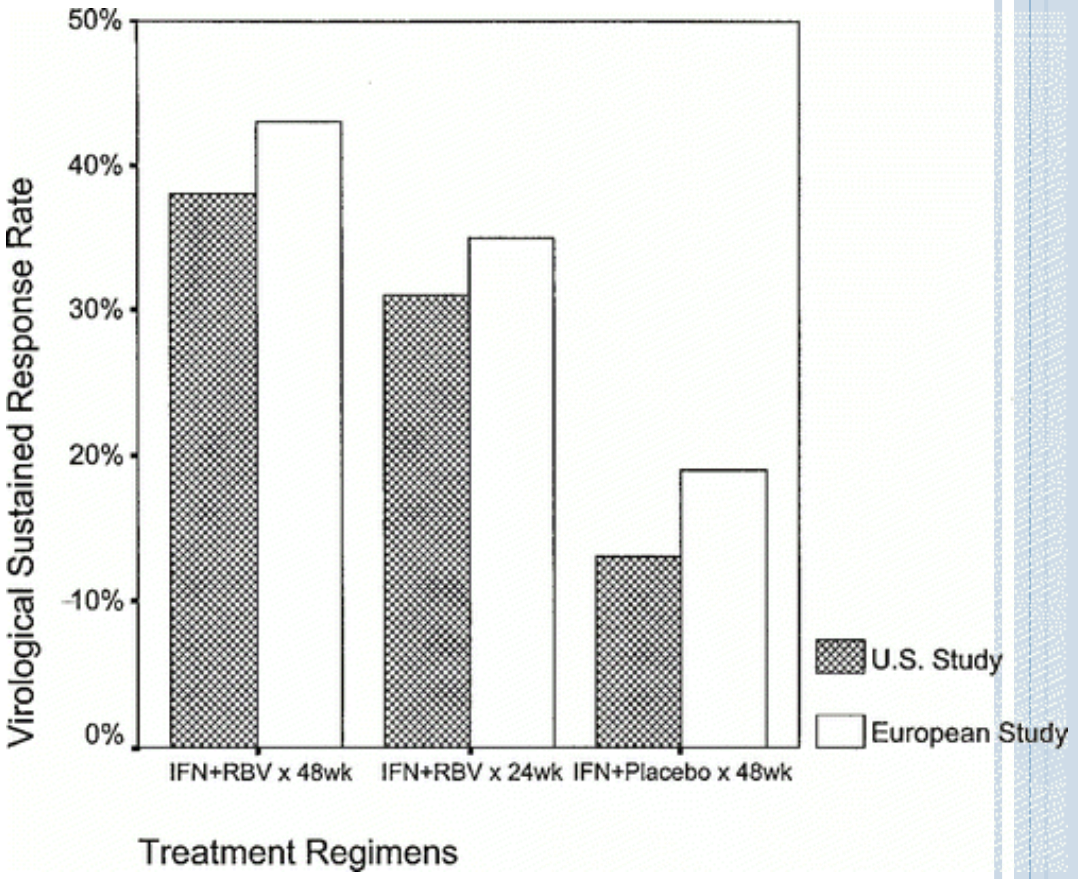
Treatment: Type I Interferon

- 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-inteferon 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

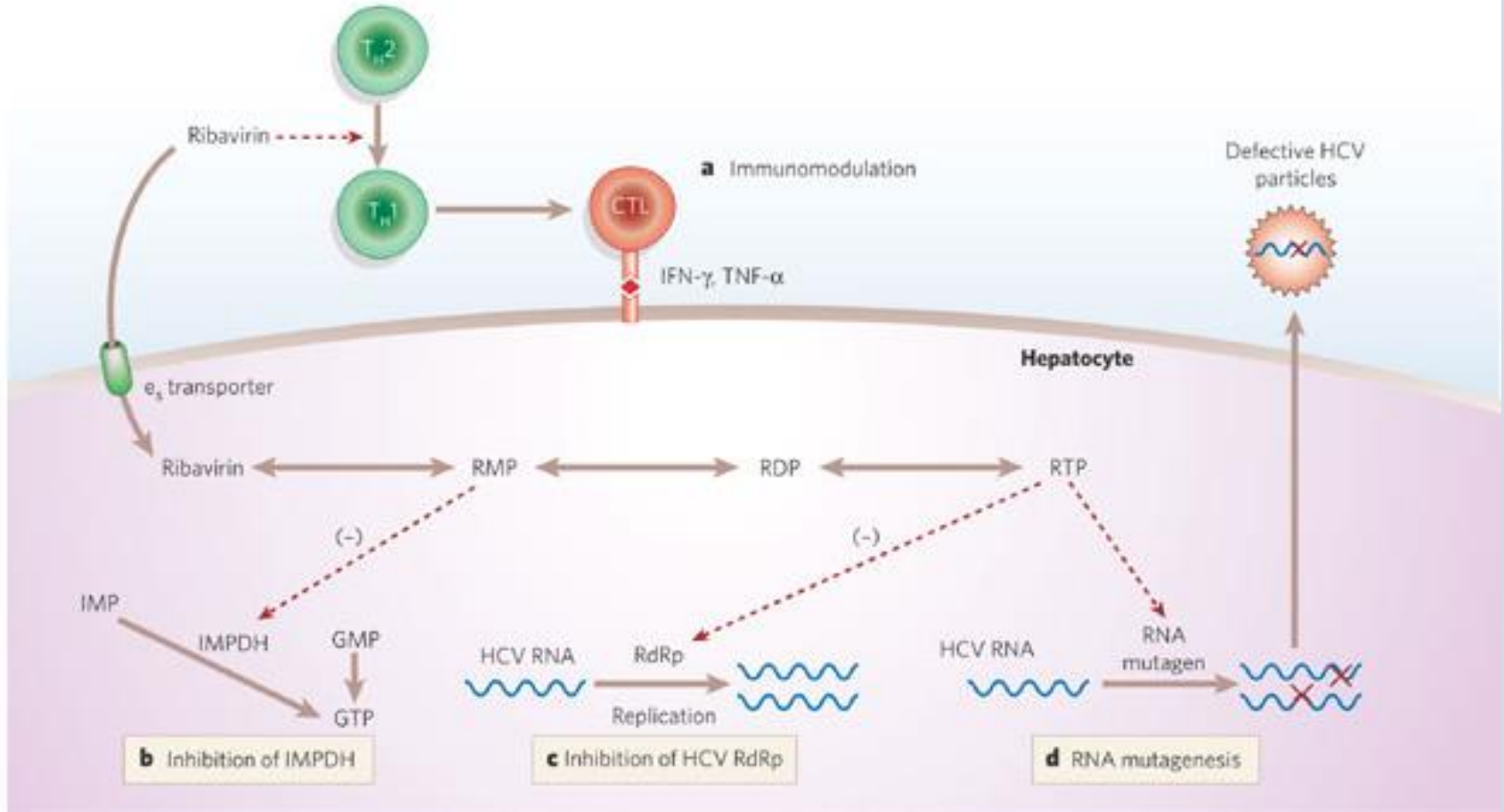
- Inteferon alone only yields a 20-25% response rate following a 12-18 month course
- Combination therapy with the "broad based" antiviral ribavirin results in 40% of individuals with SVR (30% genotype 1, 65% genotype 2 or 3)



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 hemorraghic 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

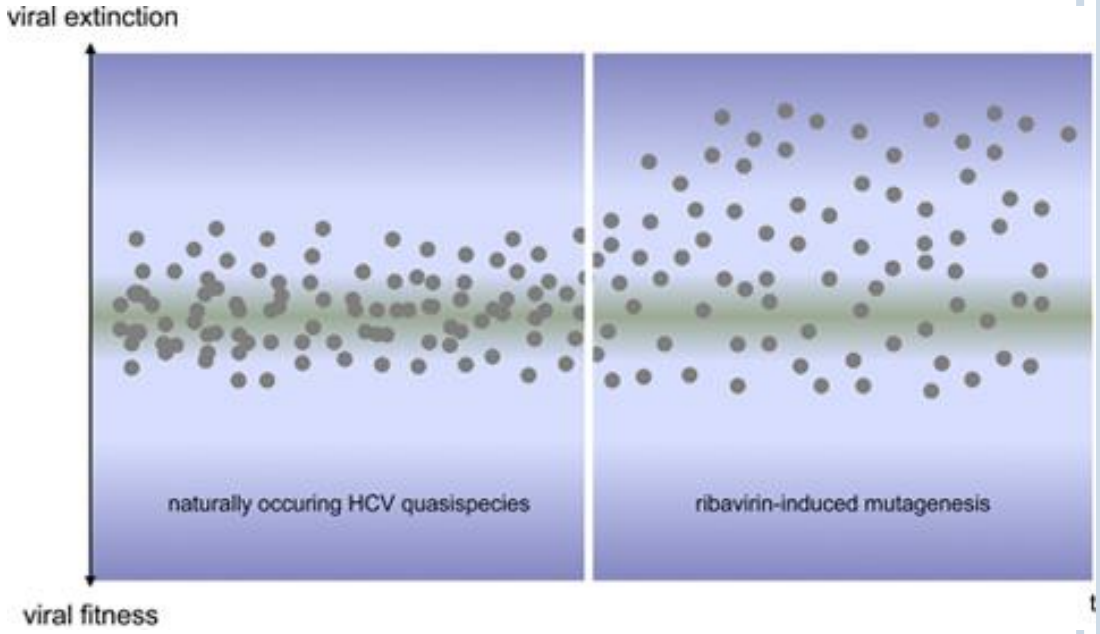
Possible Mechanisms for Ribavirin mode of action

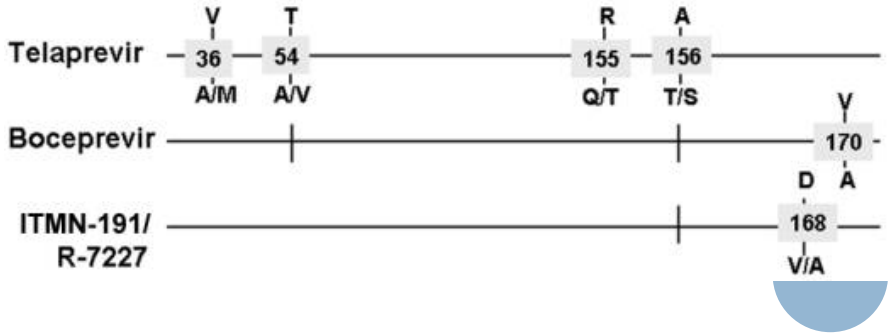


What data would help resolve ribavirin's mechanism?

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.**

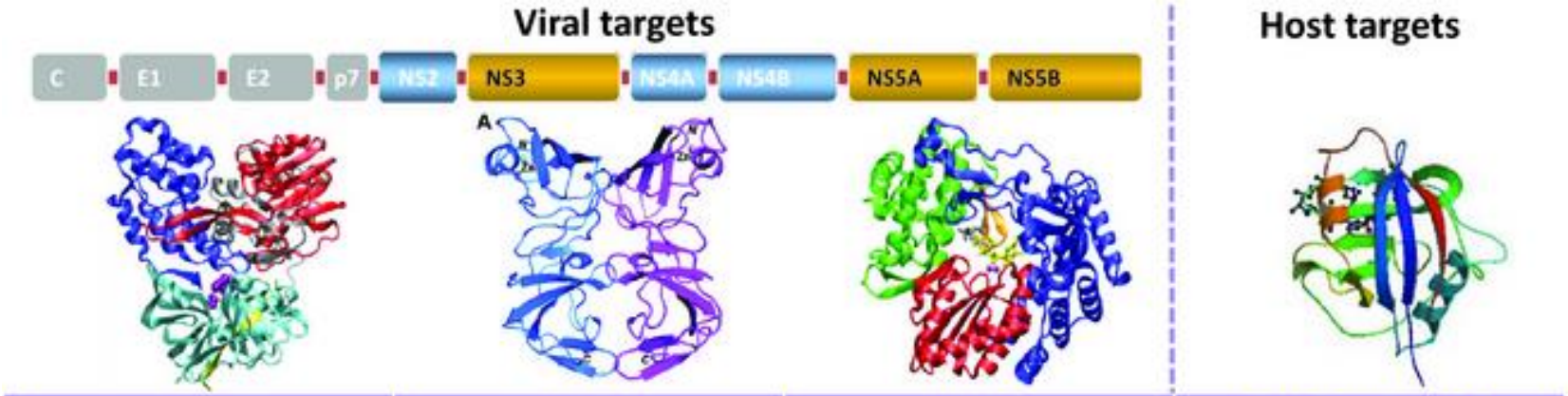




Determining an antivral treatment's mode of action

- 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 "ribarvirin function" do not recapitulate ribarvirin 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



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

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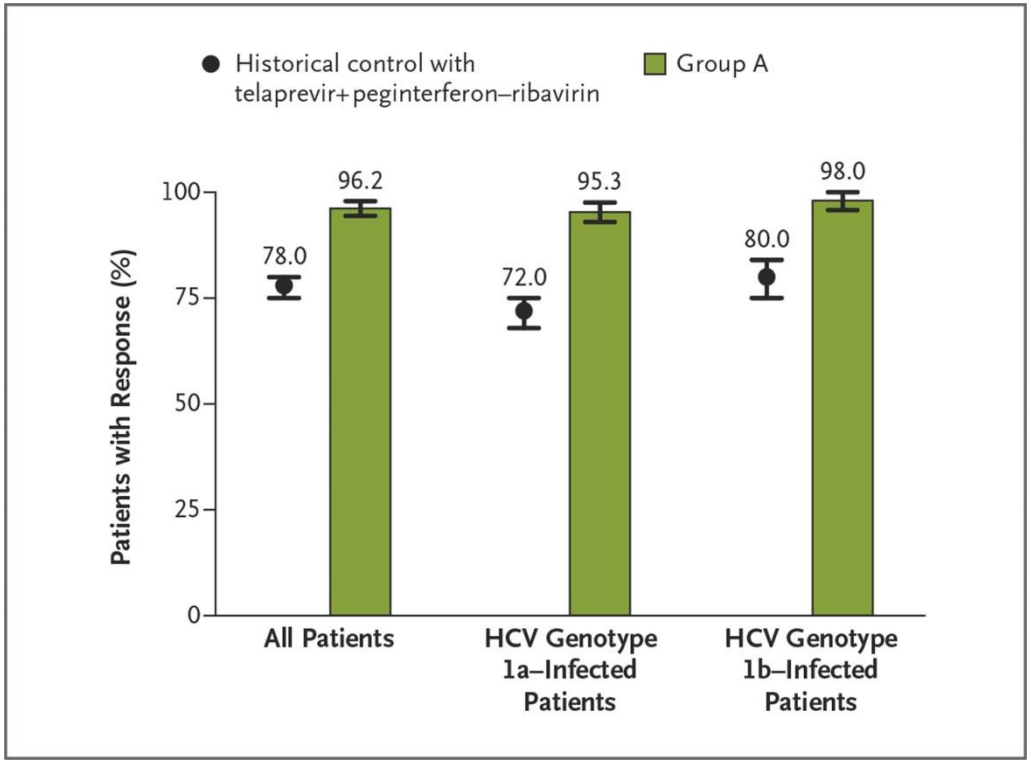
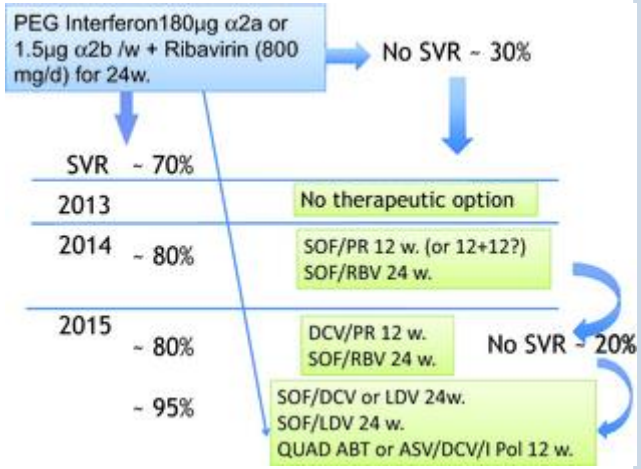
Rates of Sustained Virologic Response among All Patients and According to HCV Genotype in the Historical Control Group and in Group A.





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