associazione microbiologi clinici italiani
AMCLI
XLVI Congresso Nazionale
11-14 Novembre 2017 – Palacong

Inquadramento delle resistenze ai farmaci antivirali in tema di antimicrobial resistance

Carlo Federico Perno
ASST Grande Ospedale Niguarda

The **success of modern medicine** is mostly based on **the use of drug therapy to control or cure infections that are caused by various pathogens**, as well as other diseases such as cancer.

This legacy is currently threatened by the **development and evolution of resistance to the drugs which these therapies rely on**.

Evolutionary consequences of d resistance: shared principles acı diverse targets and organisms

Diarmaid Hughes and Dan I. Andersson

Abstract | Drug therapy has a crucial role in the treatment of viral, bacter protozoan infections, as well as the control of human cancer. The success being threatened by the increasing prevalence of resistance. We examin mechanisms of drug resistance in these diverse biological systems (usin *Plasmodium falciparum* as examples of viral and protozoan pathogens, re discuss how factors — such as mutation rates, fitness effects of resistanc and clonal interference — influence the evolutionary trajectories of dru

Mutation rates, fitness effects of resistance, epistasis and clonal interference influence the evolution of drug-resistant clones

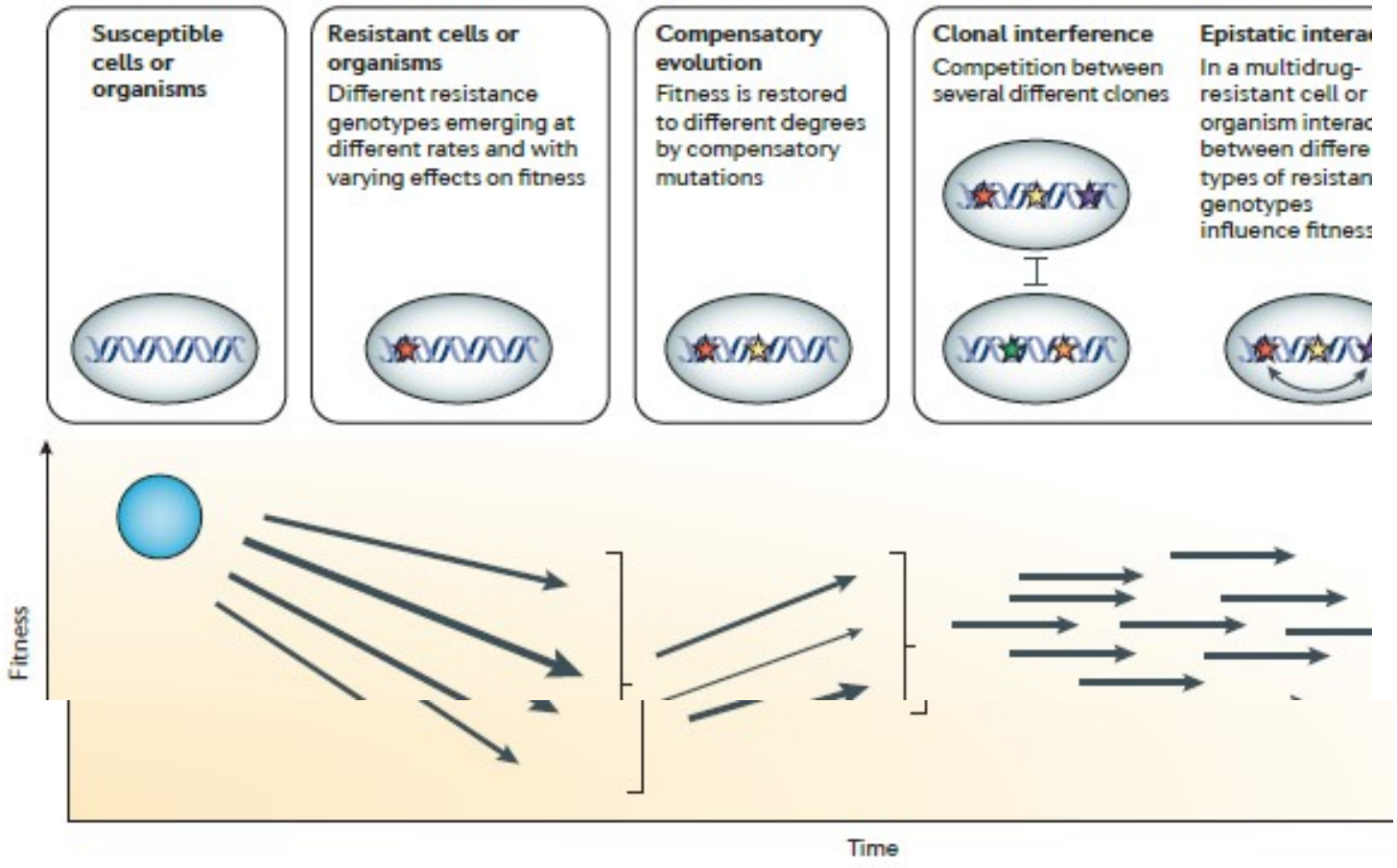


Figure 4 | Evolutionary trajectories of drug-resistance development. Evolutionary progression from a wild-type pathogen or cell type undergoing drug treatment to a drug-resistant variant is shown, displaying the effects of fitness costs of resistance, compensatory evolution, clonal interference

will gradually be lost from the population[39,40] thin arrows is not important). Multiple dif frequently arise in a population, and the enri variants will be determined by their relative

Diarmaid Hughes and Dan I. Andersson, Nature Reviews Genetics, 2015

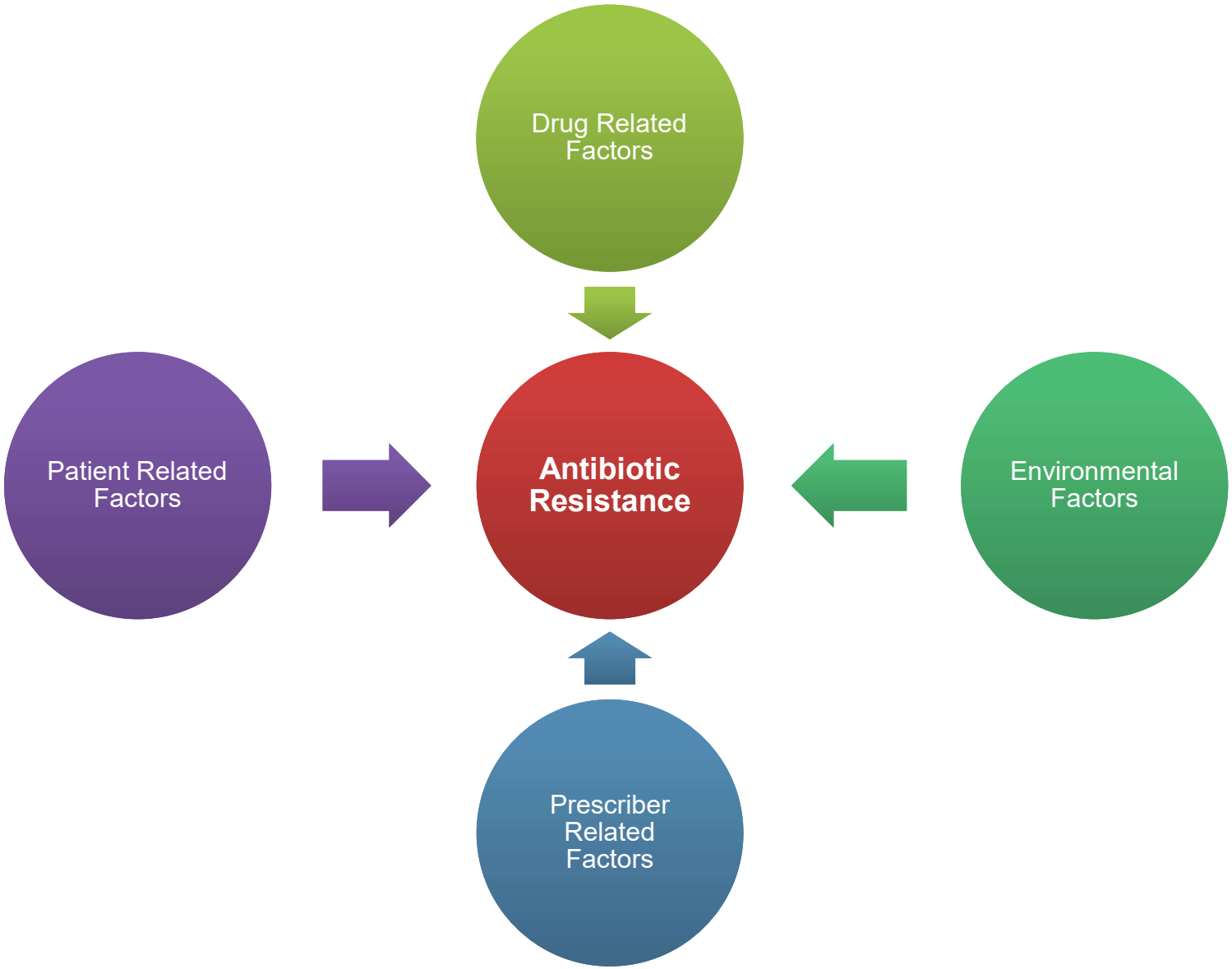
Table 1 | **Distinctions and similarities of biological systems treated with drugs to control growth and tran**

	HIV	Pathogenic bacteria	Pathogenic fungi	*Plasmodium falciparum*
Host	Human	Human, animal, plant and environmental	Human, animal, plant and environmental	Human and mosquito
Infection lifestyle	Intracellular	Extracellular (either on the host's body surface or an internal infection) and/or intracellular	Extracellular (either on the host's body surface or an internal infection), and intracellular (microsporidia)	Extracellular and intracellular
Genome size	9.7 kb, ssRNA genome	1.8 Mb (*Haemophilus influenzae*) to 6.3 Mb (*Pseudomonas aeruginosa*), dsDNA genome	Varied: ≥2.5 Mb (microsporidia), 14.8 Mb (*Candida albicans*) and 29.4 Mb (*Aspergillus fumigatus*)	22.9–26.8 Mb
Mutation rate	~10^{-5} per nt per genome replication[2,3]	~10^{-10} per nt per generation[11]. Gene amplification 10^{-2}–10^{-5} per gene per generation[13]	~10^{-10} per nt per generation (*Saccharomyces cerevisiae*)[163]	~10^{-9} per bp per generation[164]
Mechanisms of genetic diversification	Mutation and interviral recombination[4]	Mutation, genomic rearrangements and horizontal gene transfer[10]	Mutation and genomic rearrangements[35]	Mutation and genomic rearrangements[24]
In-host population size estimates	10^7–10^8 productively infected human cells[165] generating up to 10^{10} virions per day[2,3]	Highly variable depending on the pathogen and infection site. Up to 10^{11} per bladder in urinary tract infections[166]	Complicated by many fungi having a dimorphic lifestyle (yeast and hyphal forms). *Candida albicans*, ≥10^8 cfu per gram caecum in a murine infection model[167]	10^3–4×10^4 merozoites (the form that subsequently invades red blood cells) per infected liver cell[168]
Major causes of disease symptoms	HIV viral growth leads to the destruction of CD4 human immune system T cells, causing increased	Tissue damage as a result of the immune system response to bacterial growth. Many pathogenic bacteria also release toxins that directly	Tissue damage as a result of the immune system response to fungal growth. Some pathogenic fungi also release toxins that directly	Growth of *Plasmodium falciparum* within human red blood cells causes their

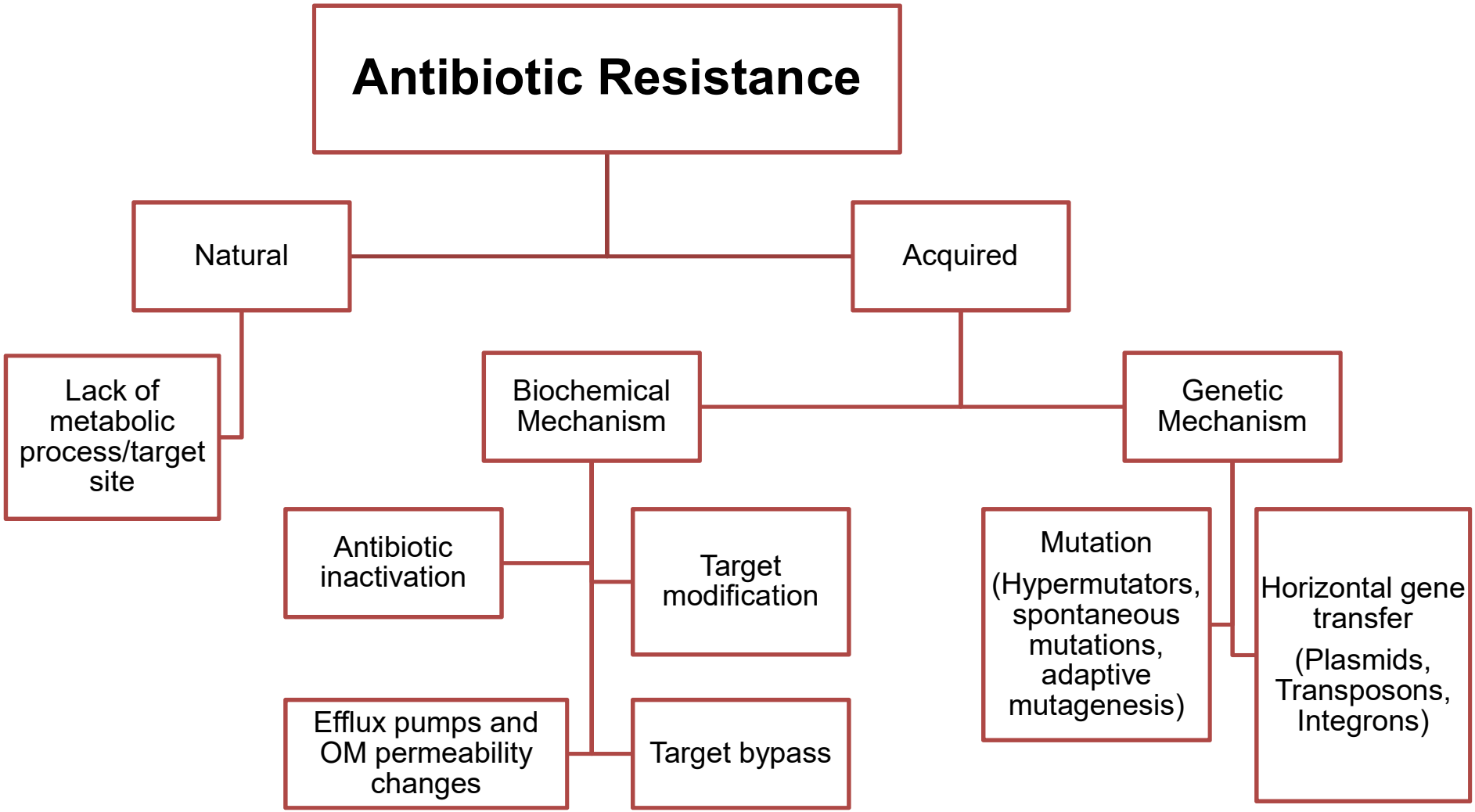
Diarmaid Hughes and Dan I. Andersson, Nature, 2015

What about the mechanisms of resistance in the pathogenic bacteria?

Factors contributing to Antibiotic Resistance

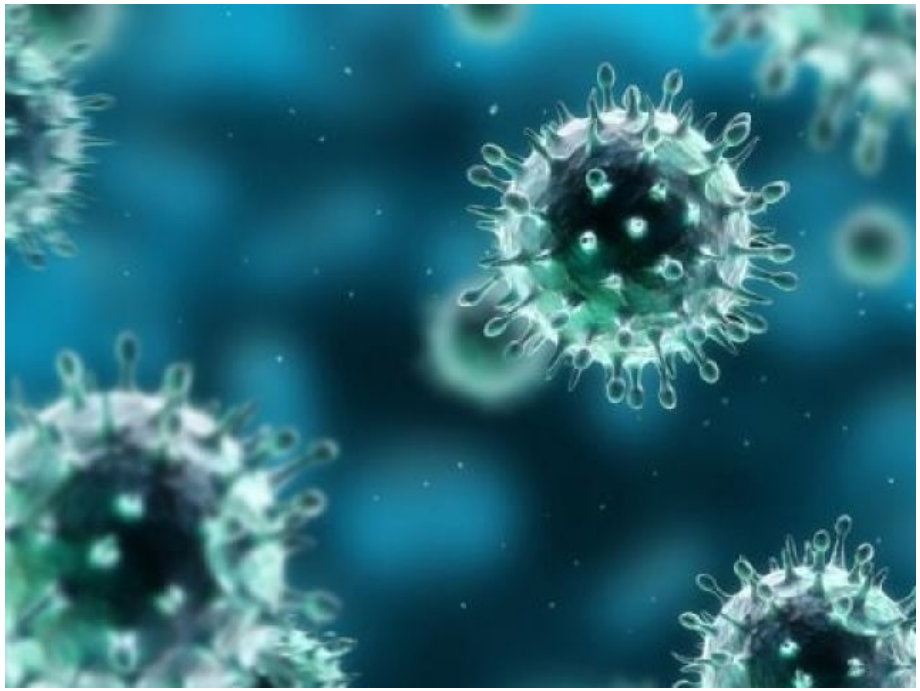


How the bacteria develops resistance?

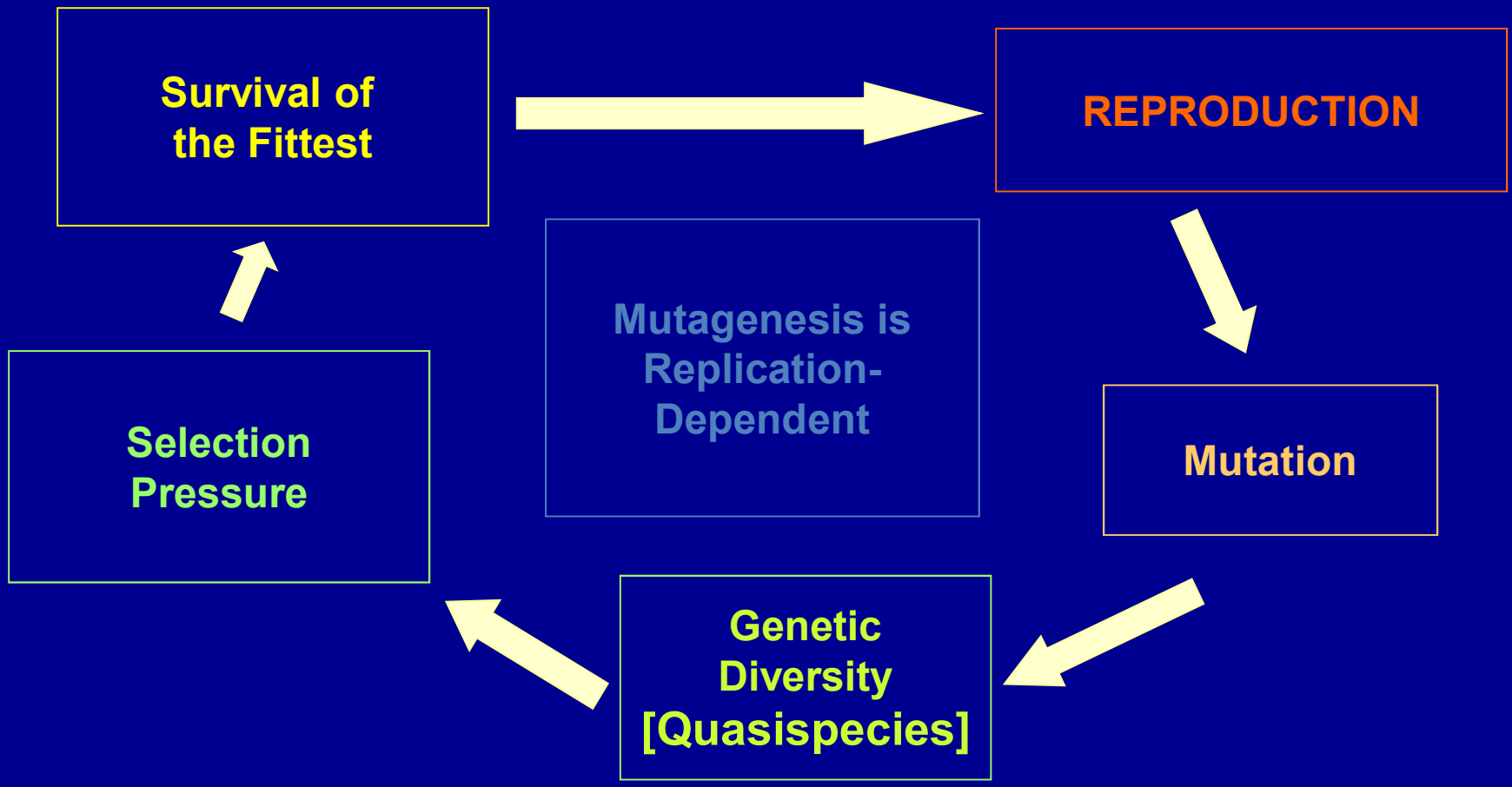


What about the mechanisms of resistance in viruses?

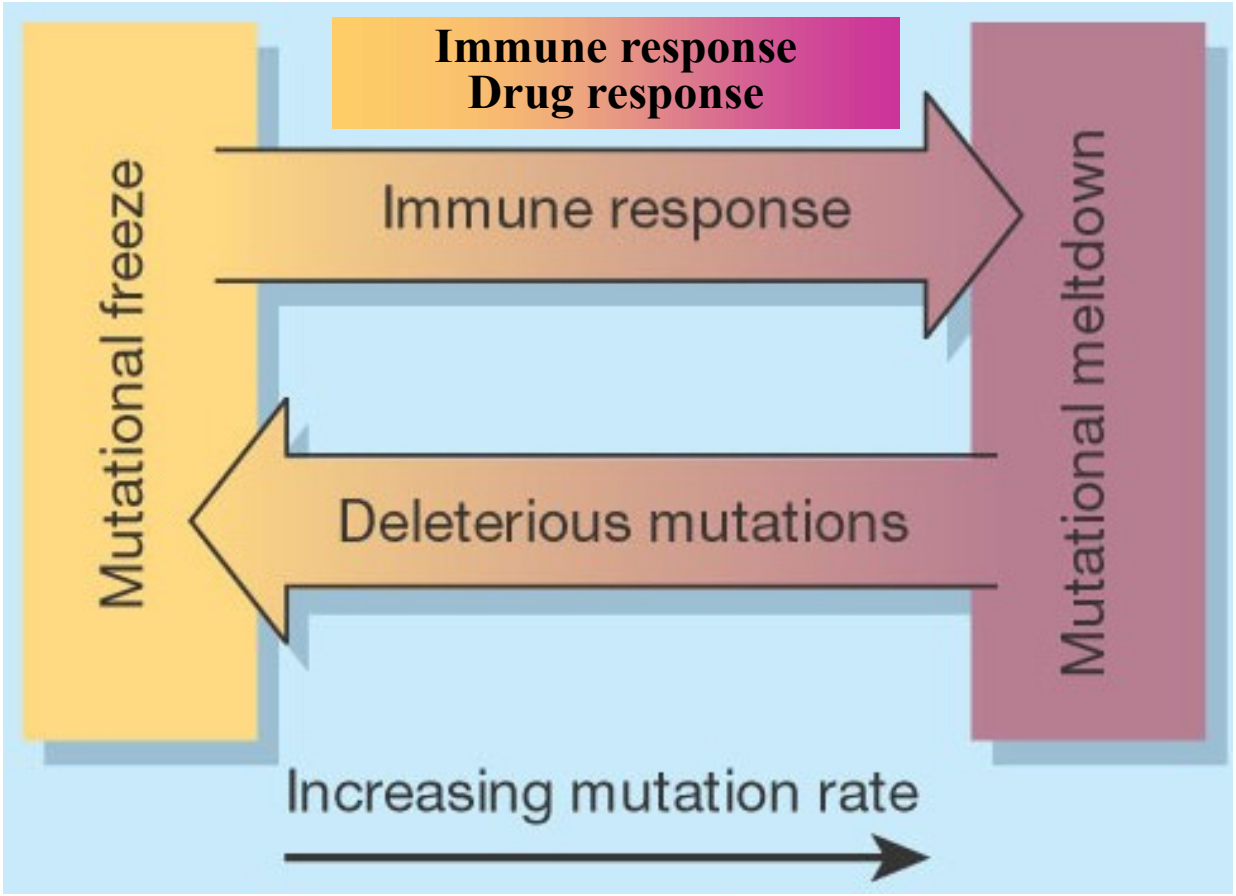
- Viruses are constantly evolving through a process that sees the alternating emergence of new genetic characteristics and their stabilization in the population by selection or random fluctuation.
- Genetic changes occur randomly during replication of the viral genome.



Darwinian Principles in Viral Evolution



Douglas D. Richman (2000) Hepatology 32:866

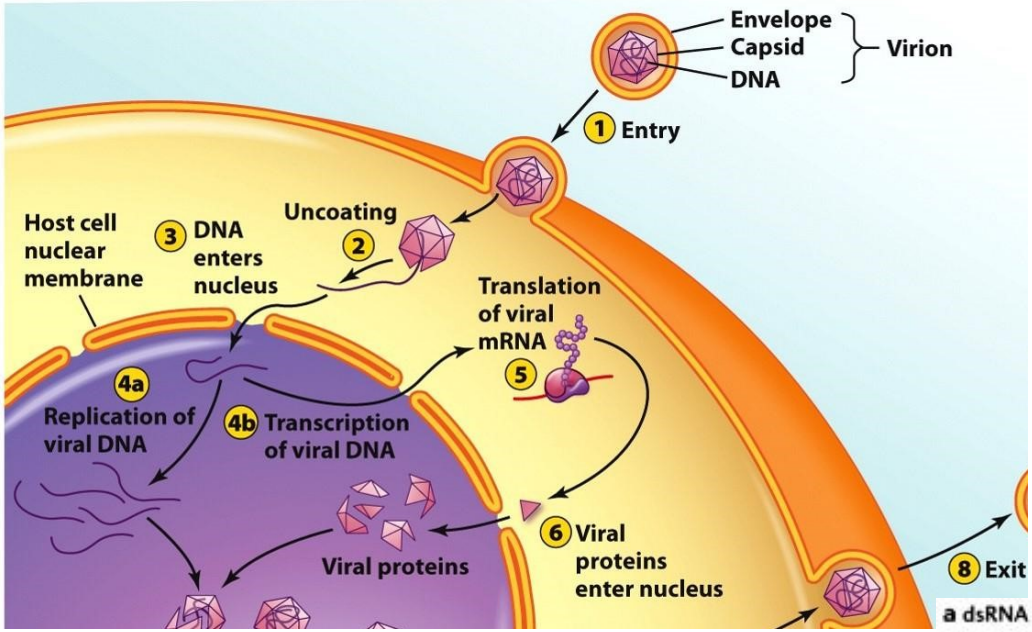


If the mutation rate is too high or too low, the viral population tends to die. This would happen because the genetic information was completely lost or because the population can not escape by the immune system responses and/or by drug pressure anymore.

Modified from Bonhoeffer et al., Nature 2002

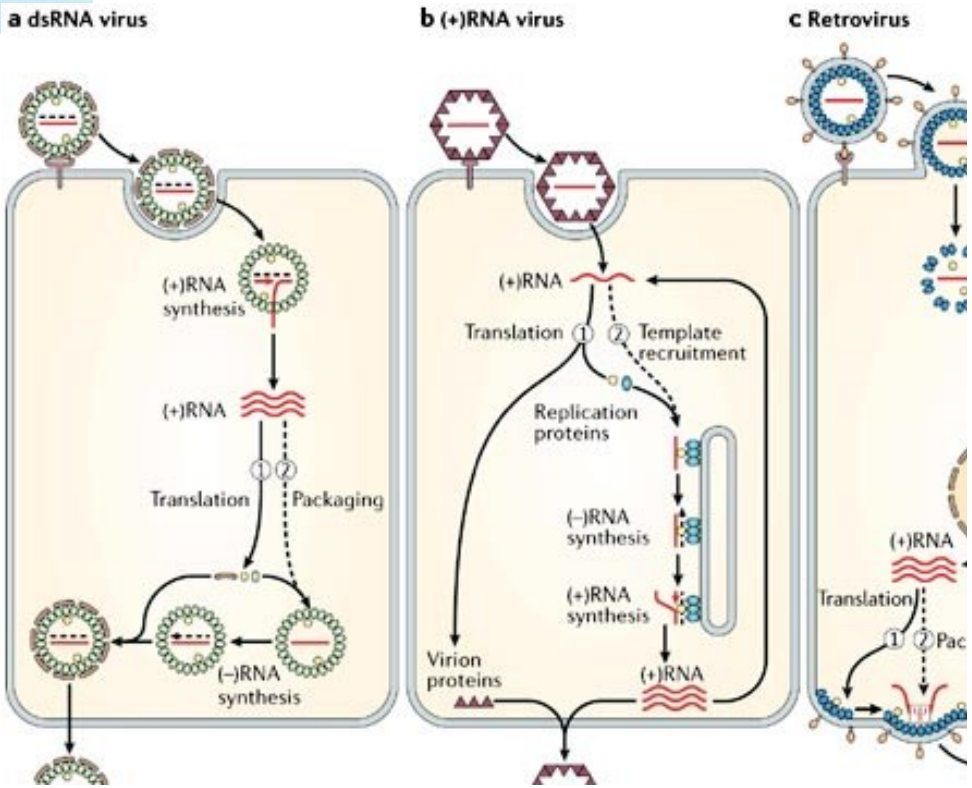
Viruses can change their genetic information through:

- Mutations
- Recombination processes



Viral replication cycle

DNA polymerase,
RNA polymerase and
reverse transcriptase
are the enzymes that copy the genetic material and are primarily responsible for creating variation in the genetic makeup of the viruses.



Point Mutations

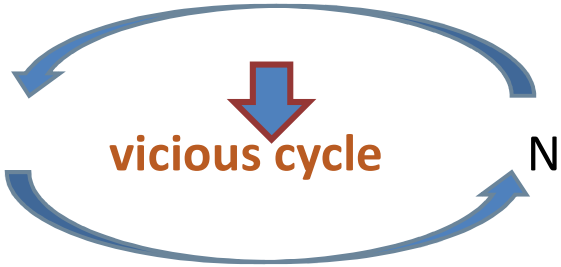
- The viruses that use **DNA-dependent DNA polymerase have a relatively low mutation rate** (1 mutation every 10^{8}-10^{11} base-pair per replication cycle), similar to that observed in cellular organisms.
- The DNA polymerase possess the ability to correct any errors made during replication of the strand (**proofreading activity**).
- The viruses that use **RNA-dependent RNA polymerases lack this proofreading activity and therefore are more prone to introduce mutations along viral genome**. In fact, the frequency of spontaneous mutations reaches 10^{-4} per nucleotide per replication cycle.
- This feature requires stringent restrictions on the extent of RNA virus genome (on average 10 times lower than that of DNA viruses) and on specific DNA virus (HBV).
- Reverse transcriptase (**RNA-dependent DNA polymerase)** of HBV and retroviruses is probably the most **error-prone** viral replicative enzyme, in the range or more of that of Viral RNA polymerases.

Often, some mutations result in changes of surface structures (antigens) that allow the virus to evade the action of host immune response (eg. neutralizing antibodies).

Virus populations that are able to escape the immune response will expand

The host immune response will, in turn, evolve trying to eliminate the new viruses

New selective pressure — **vicious cycle** — New viral antigenic variants



Related to this cascade of events, for some viruses (HIV and HCV) the concept of quasi-species was developed. The term quasispecies indicates the presence, in the same infected individual the presence of a swarm of genetically different viral variants.

Viruses can change their genetic information through:

- Mutations
- **Recombination processes**

Viral Recombination and Reassortment

Recombination and **Reassortment** involve the exchange of genetic material between two different viruses of the same species during coinfection of a host cell, with the consequent generation of a viral progeny with intermediate characteristics between the two parental viruses.

If the new characteristics confer a selective advantage, the recombinant viruses are favored by selection!

Recombination happens frequently in DNA viruses, but also in RNA viruses (mixoviruses, coronaviruses, retroviruses).

Reassortment can occur in viruses with a segmented genome (orthomixoviruses, reoviruses, ..).

The case of HIV

- At every replication cycle, changes are made in viral genome

"**Mutations**"

- RT enzyme has an error rate during transcription of 1:2,000-10,000 bases.
- The genome of HIV is made of 9749 nucleotides.
- Therefore, **each** new virus can have one mutation in its genome!

The population of viral variants in the same individual is highly heterogeneous

- The accumulation of mutations in HIV (*env* and also *gag* genes) makes the virus less susceptible to humoral and cellular responses of the immune system.

- **This represents a major obstacle for the development of a specific immunity against HIV-1**
  - **Up to day, no vaccines are available against HIV, and nothing on the close horizon**
  - **Up to day, the production of broadly neutralizing antibodies against endogenous HIV is limited to a restricted number of HIV-infected patients, that normally are characterized by a limited (if not absent) progression of the disease**

HIV-1 env gene

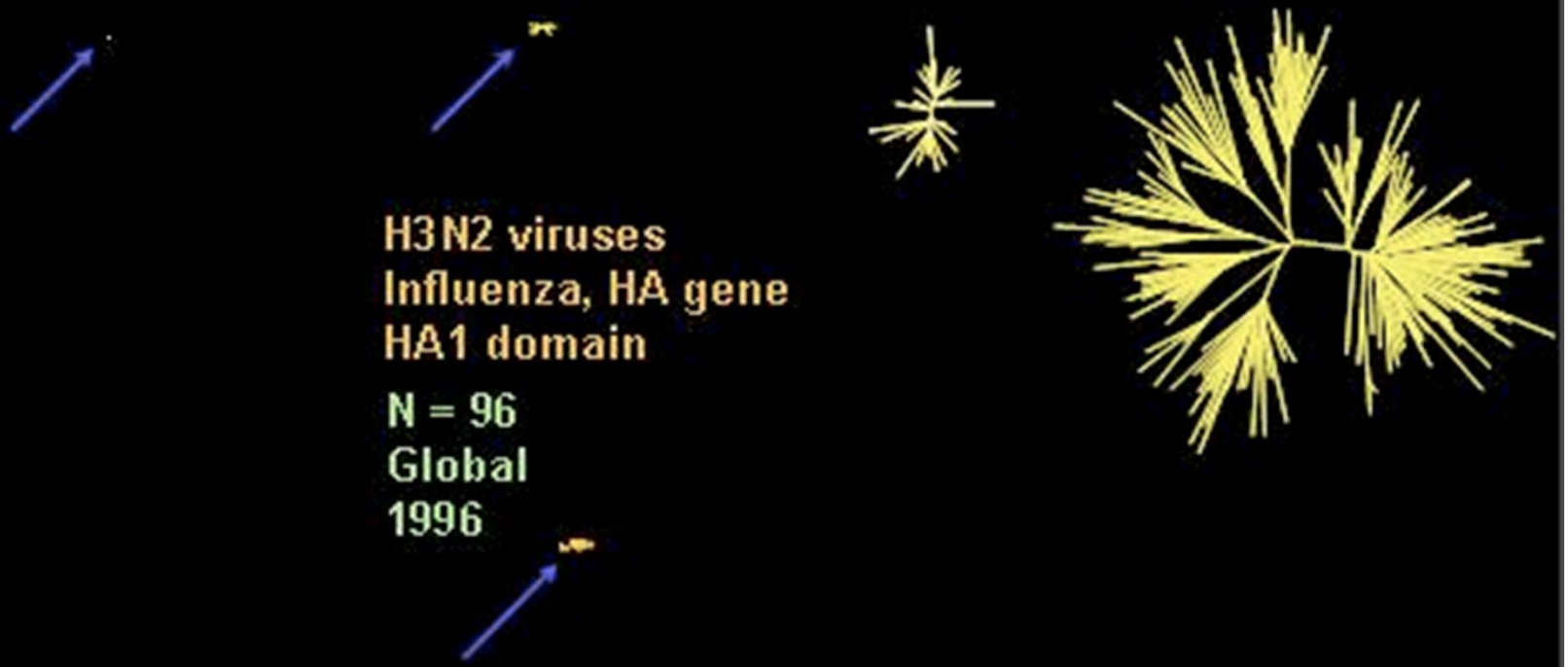
Korber *et al., Brit Med Bull*, 2001

N = 26
Kaliningrad
CRF03_AB
1996-1997

N = 9
Single individual
6 years
post infection

N = 23
Amsterdam
Subtype B
1990-1991

N = 193
DRC
Group M
1997

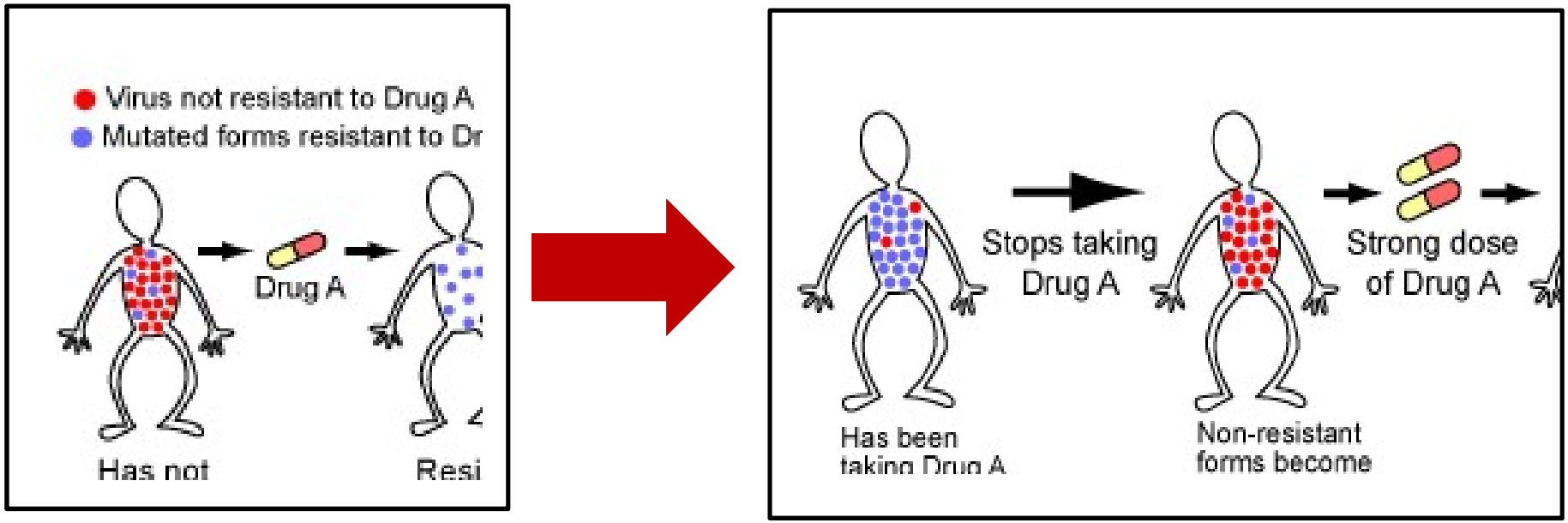


H3N2 viruses
Influenza, HA gene
HA1 domain

N = 96
Global
1996

HIV evolves so quickly that it evolves right under our treatments.

Because of HIV's speedy evolution, it responds to selection pressures quickly: viruses that happen to survive the drug are favored, and resistant virus strains evolve within the patient, sometimes in just a few days/weeks.

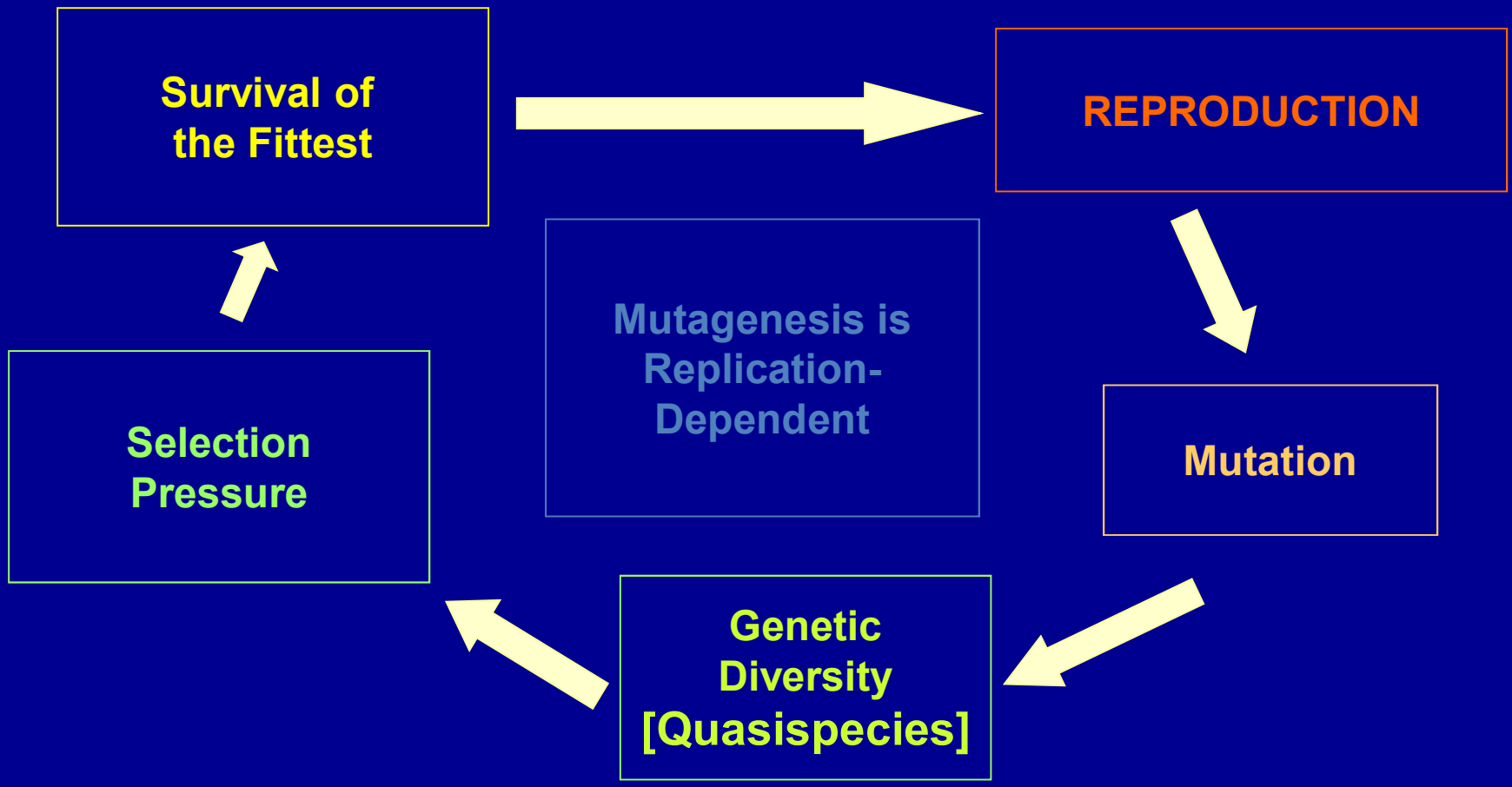


HIV Genetic Variability

When the drug pressure is sub-optimal :

- The virus escapes to antiviral drugs through the accumulation of several specific and well known mutations.
- With some exceptions, the resistance to anti-HIV drugs is mostly generated by suboptimal pressure by antiviral drugs, that favours the selection and fixation of resistant strains provided with high fitness

Darwinian Principles in Viral Evolution



Douglas D. Richman (2000) Hepatology 32:866

If today were November 13, 1989 ….

Drug-resistant strains of AIDS virus found [news]

Science. 1989 Mar 24;243(4898):1551-2. Unique Identifier : AIDSLINE MED/89186893

Marx JL

Keywords: Acquired Immunodeficiency Syndrome/*DRUG THERAPY *Drug Resistance, Microbial Human HIV/*DRUG EFFECTS Z

890730

Science 1 December 1989:
Vol. 246 no. 4934 pp. 1155-1158
DOI: 10.1126/science.2479983

Multiple mutations in HIV-1 reverse transcriptase confer high resistance to zidovudine (AZT)

BA Larder and SD Kemp

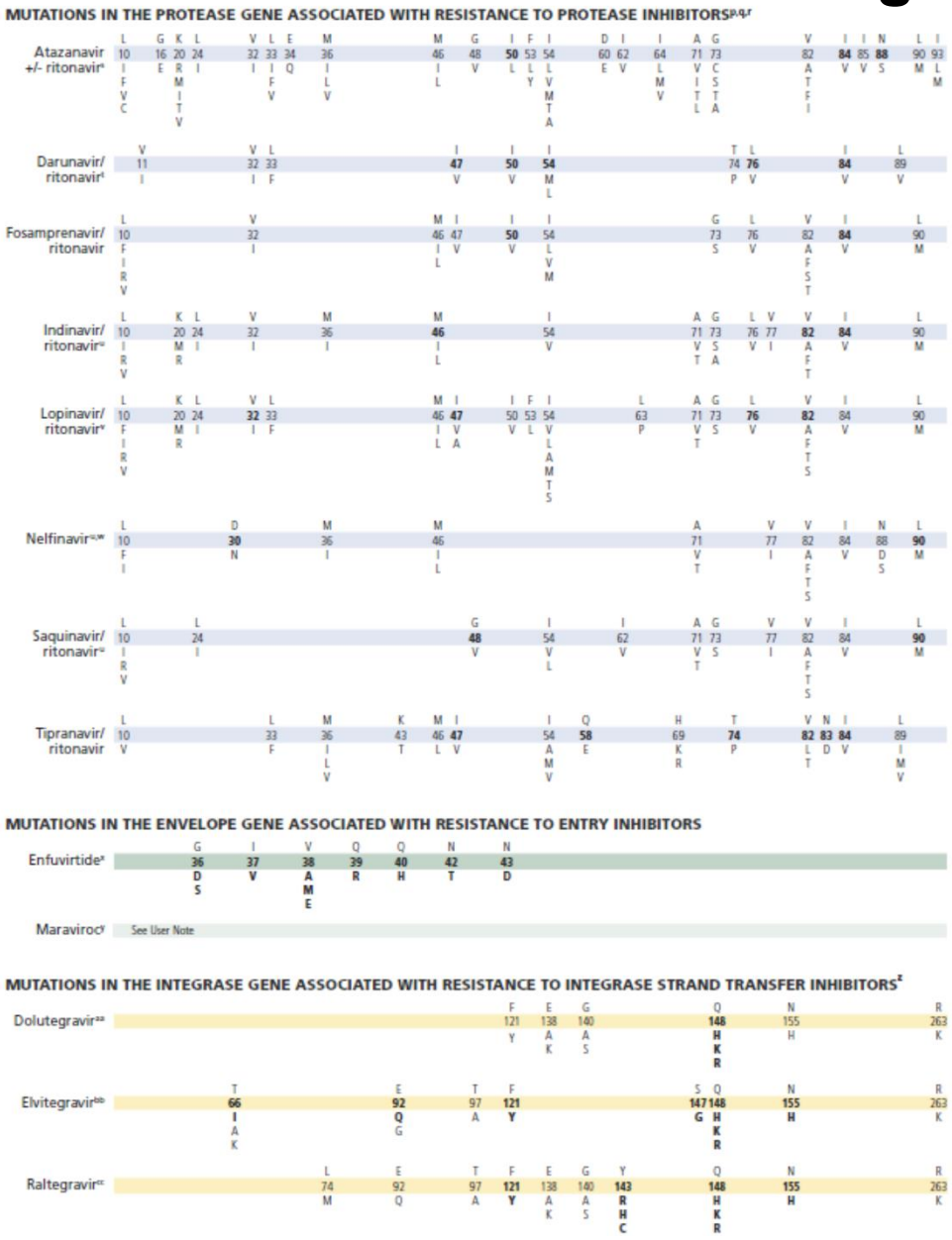
± Author Affiliations

ABSTRACT

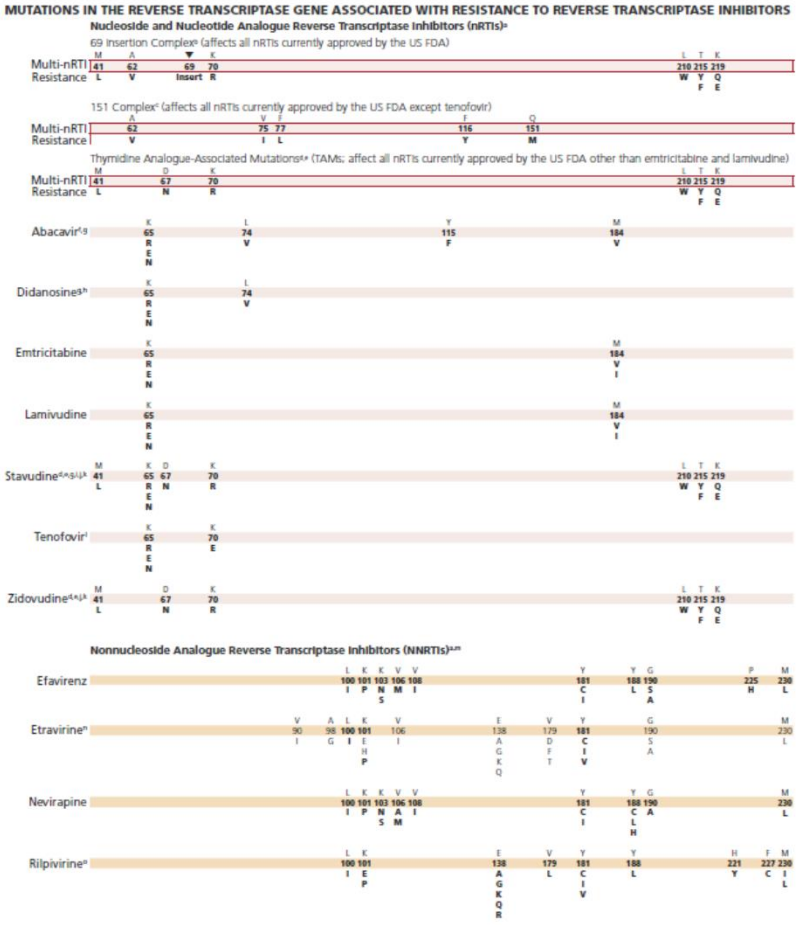
Human immunodeficiency virus (HIV) isolates with reduced sensitivity to zidovudine (3'-azid 3'-deoxythymidine, AZT) from individuals with acquired immunodeficiency syndrome (AIDS) complex were studied to determine the genetic basis of their resistance. Most were seque obtained at the initiation of and during therapy. Comparative nucleotide sequence analysis transcriptase (RT) coding region from five pairs of sensitive and resistant isolates identifie amino acid substitutions common to all the resistant strains (Asp67----Asn, Lys70----Arg, T plus a fourth in three isolates (Lys219----Gln). Partially resistant isolates had combinations changes. An infectious molecular clone constructed with these four mutations in RT yielde HIV after transfection of T cells. The reproducible nature of these mutations should make it

4 RT mutations were associated with drug-resistance

Today more than 100 mutations have been associated with drug resistance



IAS Dec 2016/Jan 2017



Wensing AM, et al. Top HIV Medicine 2017

By the arrival of the new wave of integrase inhibitors, a recruitment for revising resistance monitoring is crucial today

Knowledge of HIV-1 resistance is continuously evolving

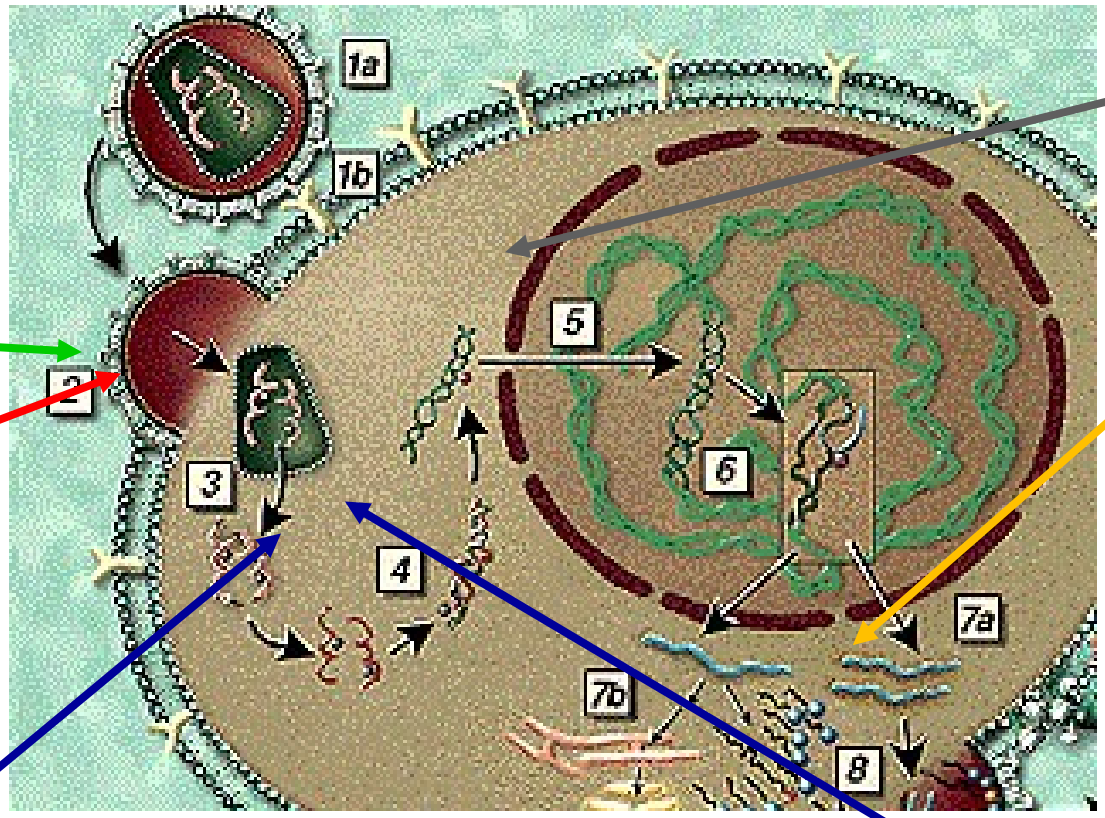
IAS Dec 2016/Jan 2017

	T	L	E	T	F	E	G	Y	S	Q	N	R
Dolutegravir[aa]		74?		97?	121	138	140	143?		148	155	263
		I		A	Y	A	A	C		H	H	K
						K	S			K		
										R		
Elvitegravir[bb]	66		92	G	F	E	G	Y	147	148	155	263
	I		Q	118?	121	138?	140?	143?	G	H	H	K
	A		G	R	Y	A	A	C		K		
	K					K	S			R		
Raltegravir[cc]		L	E	G	F	E	G	Y		Q	N	R
		74	92	118?	121	138	140	143		148	155	263
		M	Q	R	Y	A	A	R		H	H	K
						K	S	H		K		
								C		R		

Wensing AM, et al. Top HIV Medicine 2017

Almost every step of HIV replication is target of at least one drug

(1) Binding & fusion – (2) Entry – (3) Uncoating – **(4) Reverse transcription – (5) Integration** – (6) Transcription – (7) Translation - (8) Assembly & budding – **(9) Maturation**



3 Integrase inhibitors (INIs)
RAL, EVG, DTG

***1** Entry inhibitor:*
MVC

***10** Protease inhibitors (PIs)*
SQV, IDV, RTV, NFV, APV, FPV, LPV, ATV, TPV, DRV

1** Fusion inhibitor (FIs): **T20

8 Nucleoside reverse transcriptase inhibitors (NRTIs):
AZT, ddI, ddC, d4T, 3TC, ABC, TDF, TAF, FTC

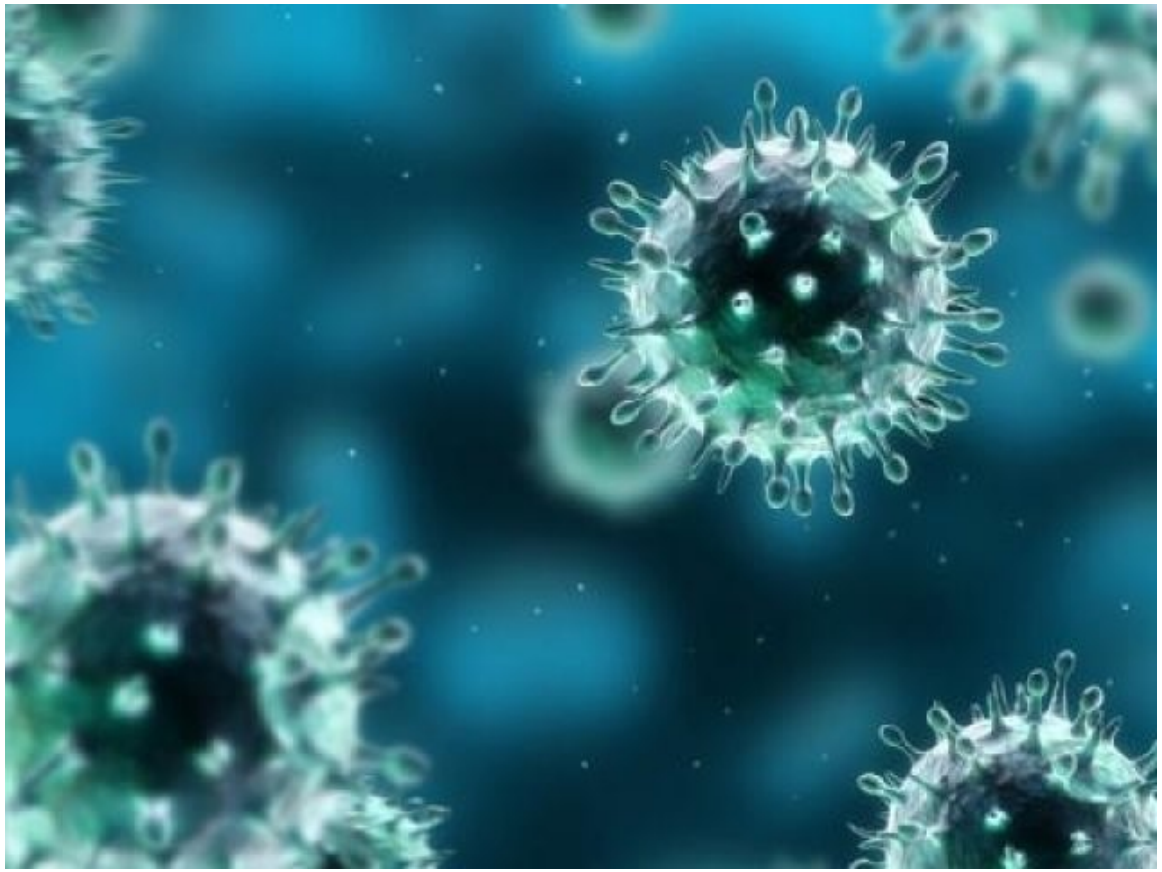
***5** Non-nucleoside reverse transcriptase inhibitors (NNRTIs)*
EFV, NVP, DLV, ETR, RPV

The case of HCV

Hepatitis C: 28 years from its discovery, and 150,000 from its first contact with humans

Science AAAS

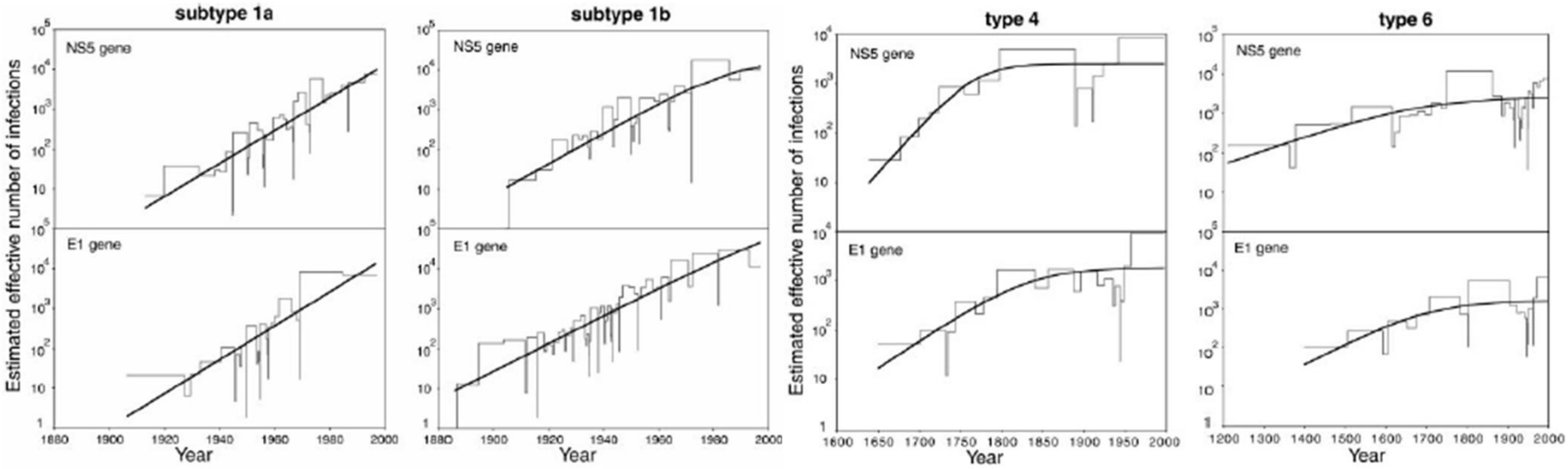
The HCV team (left to right; *M. Houghton, Q.L. Choo, G. Kuo e D. Bradley*)



The origin of the primate Flaviviridae could be as ancient as the differentiation of primate species some 35 million years ago

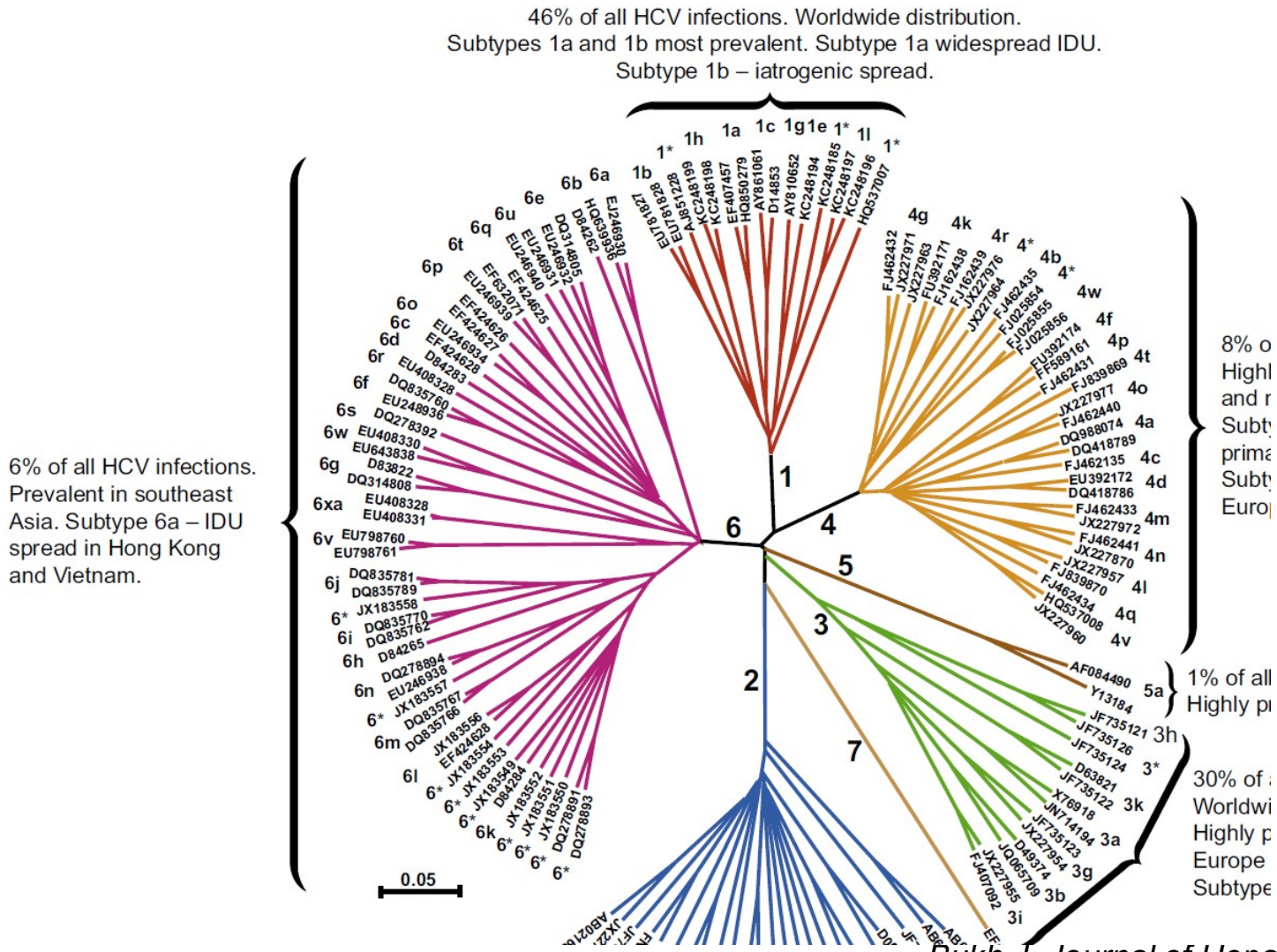
HCV could have been coevolving with human populations during their migration out of Africa within the past 100,000 to 150,000 years, **but the current HCV genotypes appeared much more recently**.

A study suggested that types 6 and 4 could have originated 700 years and 350 years ago, respectively, whereas **subtypes 1a and 1b could have arisen less than 100 years ago.**



Pybus et al Science 2001

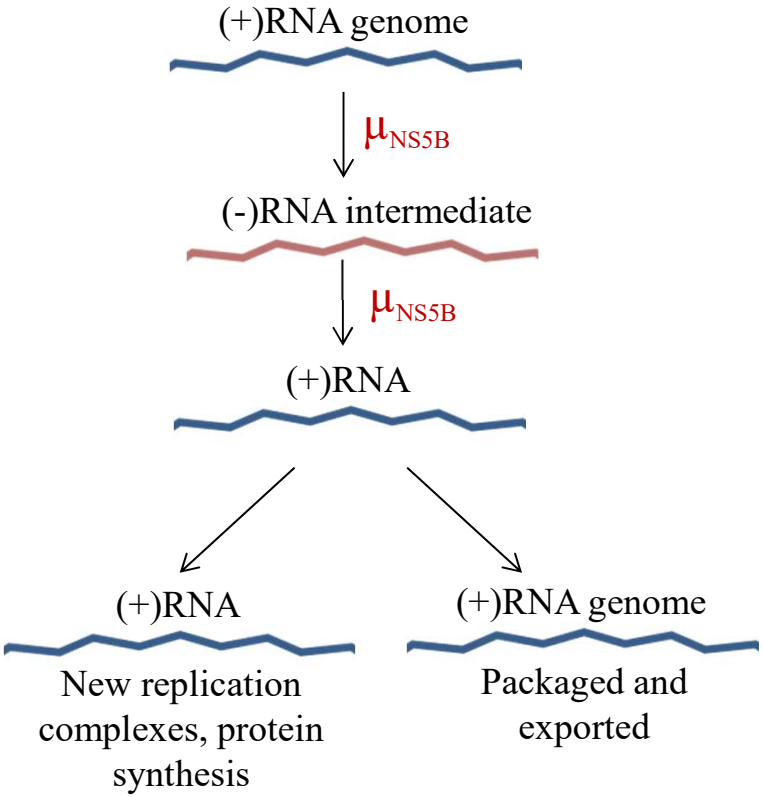
Similarly to HIV, the extreme genetic variability of HCV is the major obstacle for the development of vaccines to this virus.



Bukh J, Journal of Hepatology 2016

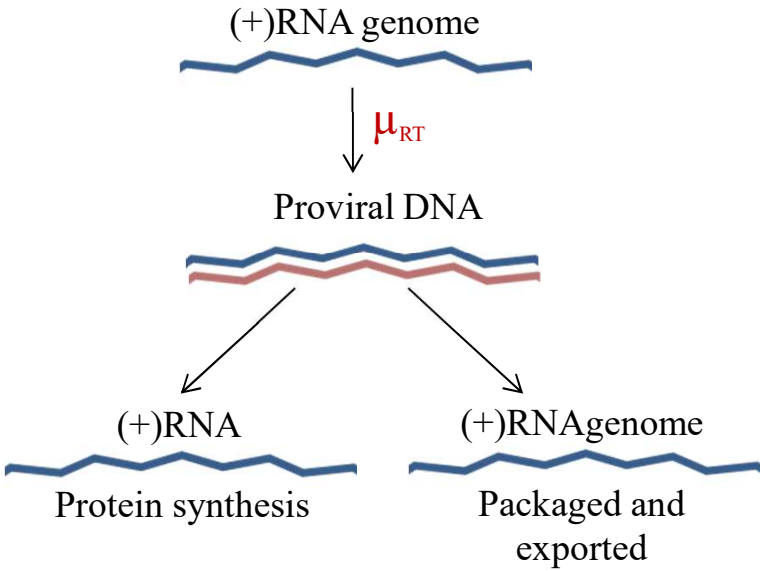
HCV

Double passage by viral NS5B polymerase to create a (+)RNA template for protein synthesis



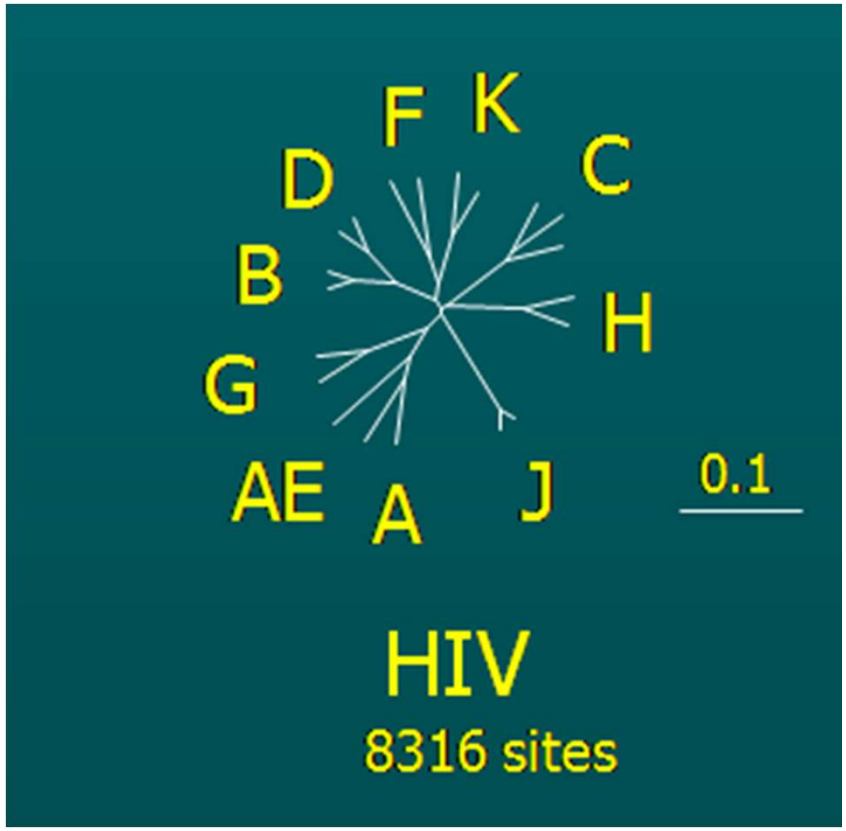
HIV

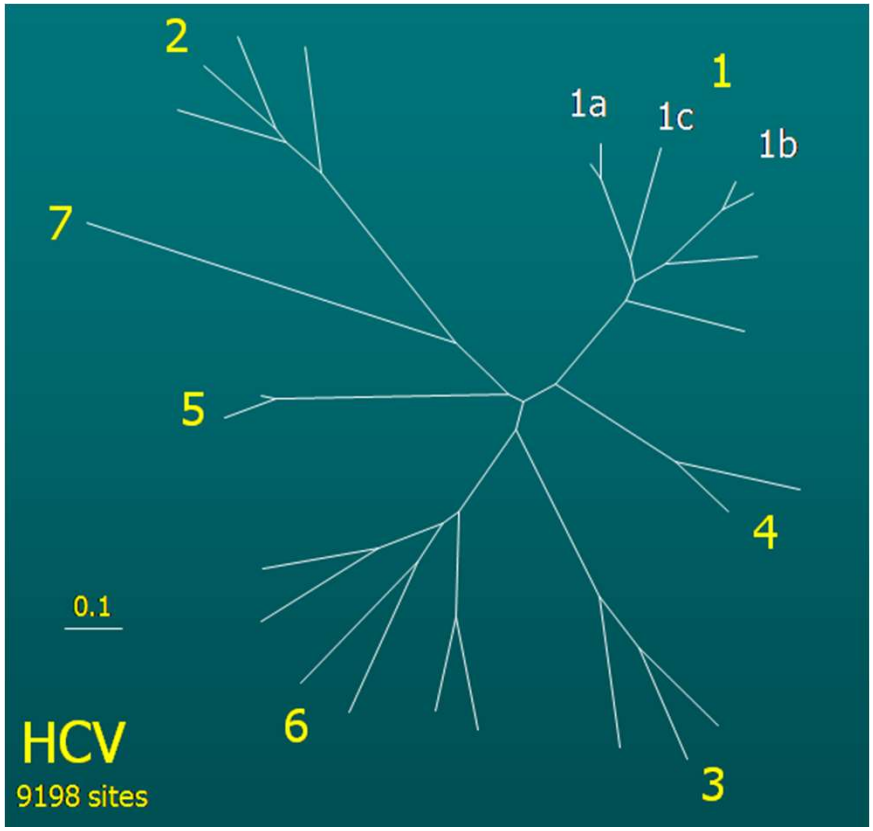
Single passage by viral reverse-transcriptase (RT) to create proviral DNA and then a (+)RNA template for protein synthesis



Regardless of NS5B/RT mutational rates, quasispecies complexity is higher in HCV in respect to HIV ...

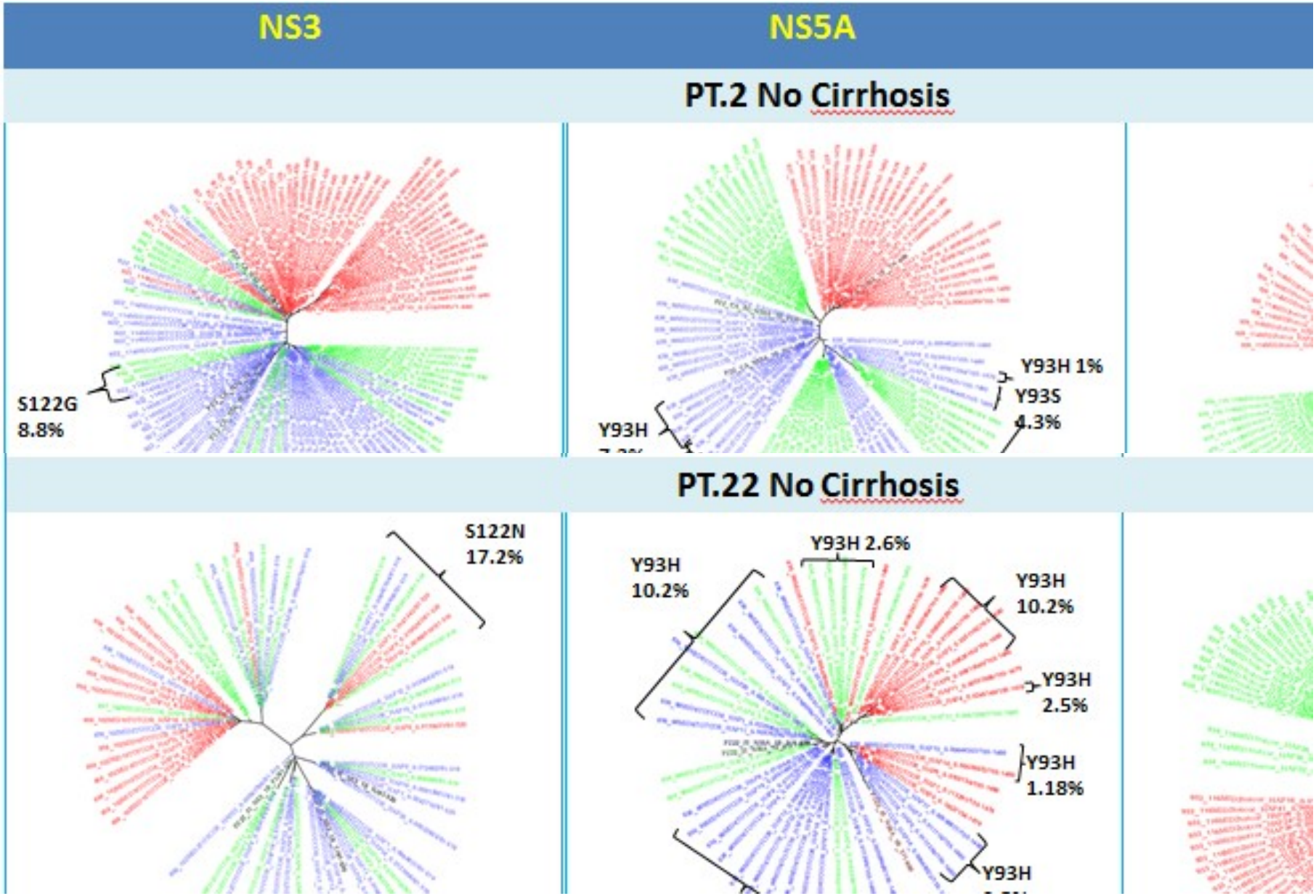
HCV genetic variability is higher than HIV





31%–33% nucleotide difference among the 7 known HCV genotypes and 20%–25% among the nearly 67 HCV subtypes (Smith et al., 2014).

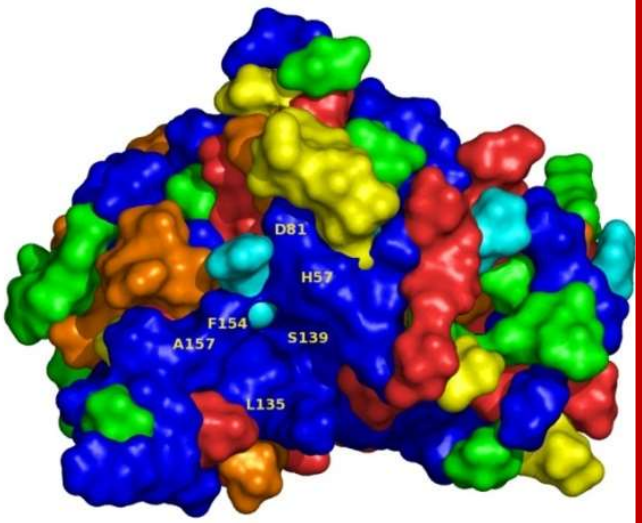
Phylogenetic trees showing individual clusters among and within the same compartments, for all genes (more evident for NS5A/NS5B), exclusively in the non cirrhotic patients



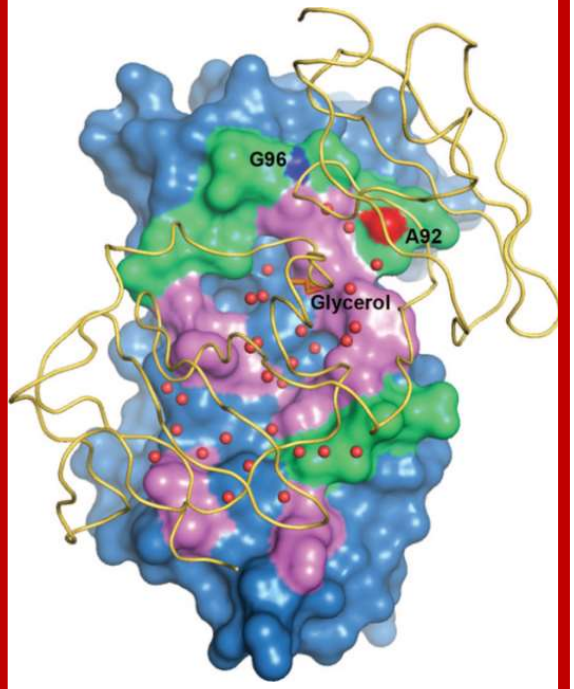
UDPS sequences: in red tumoral tissue; blu non-tumoral and green plasma compartments

Sorbo et al., EASL 2016

Any of the 3 DAA-target HCV proteins can present natural resistance associated substitutions (RASs)

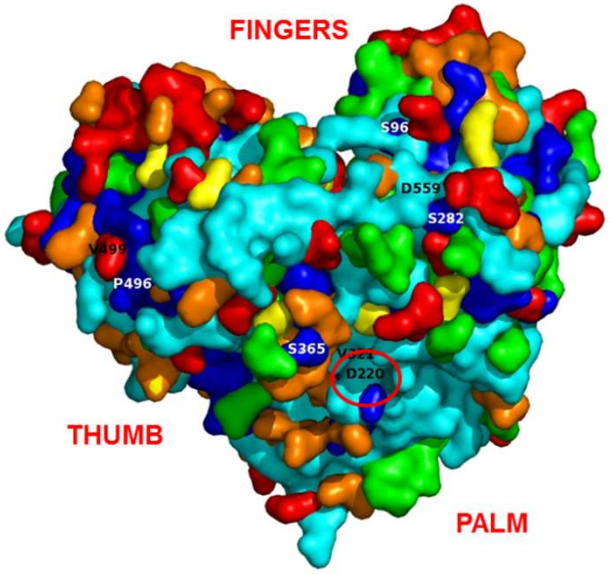


47% amino acids of HCV PROTEASE NS3 are conserved among all HCV-genotypes



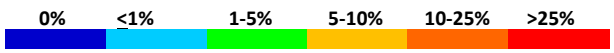
46% amino acids of HCV NS5A are conserved among all HCV-genotypes

NS5A



55% amino acids of HCV POLYMERASE NS5B are conserved among all HCV-genotypes

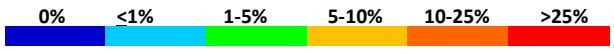
Amino acid variability:



Cento et al., PLoS ONE 2012

Love et al., J Vir 2009

Amino acid variability:



Di Maio et al., AAC 2014

EASL 2016

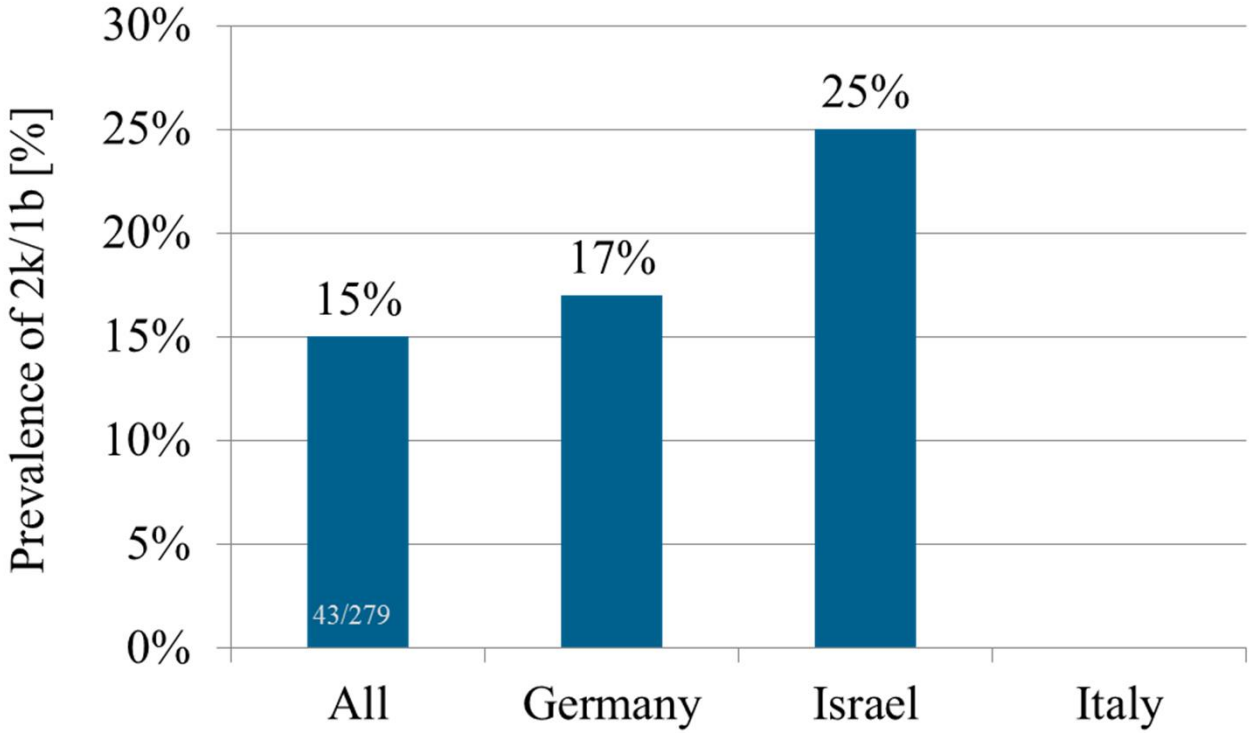
UNIVERSITÄTS KLINIKUM FRANKFURT
GOETHE-UNIVERSITÄT

PREVALENCE AND CLINICAL IMPORTANCE OF HEPATITIS C VIRUS (HCV) GENOTYPE 2K/1B CHIMERAS

Simone Susser[1], Julia Dietz[1], Bernhardt Schlevogt[2], Mira Barak[3], Rasha Daniel[3], Valeria Piazzolla[4], Sandra Passmann[1], Holger Hinrichsen[5], Markus Cornberg[2], Eli Zuckerman[6], Alessandra Mangia[4], Stefan Zeuzem[1], Christoph Sarrazin[1]

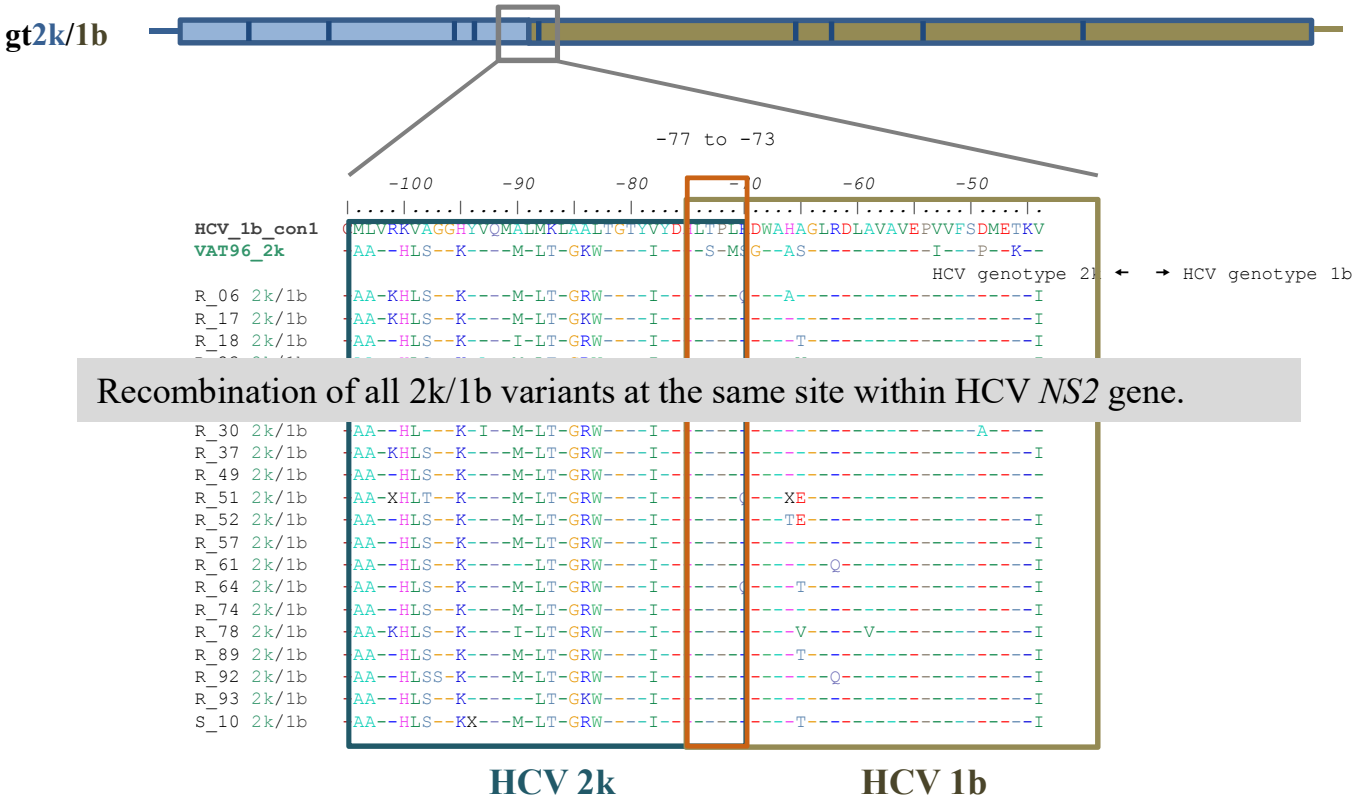
[1]Goethe-University Hospital, Medical Clinic 1, Frankfurt, Germany; [2]Hannover Medical School, Department of Gastroenterology, Hepatology and Endocrinology, Hannover, Germany; [3]Haifa and Western Galilee Laboratory, Clalit Health Services, Nesher, Israel; [4]Liver Unit, IRCCS Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo, Italy; [5]Gastroenterology, Gastroenterologische Schwerpunkt Praxis, Kiel, Germany; [6]Liver Unit, Carmel Medical Center and Rappaport Faculty of Medicine, The Technion, Haifa, Israel

Prevalence of 2k/1b recombinants



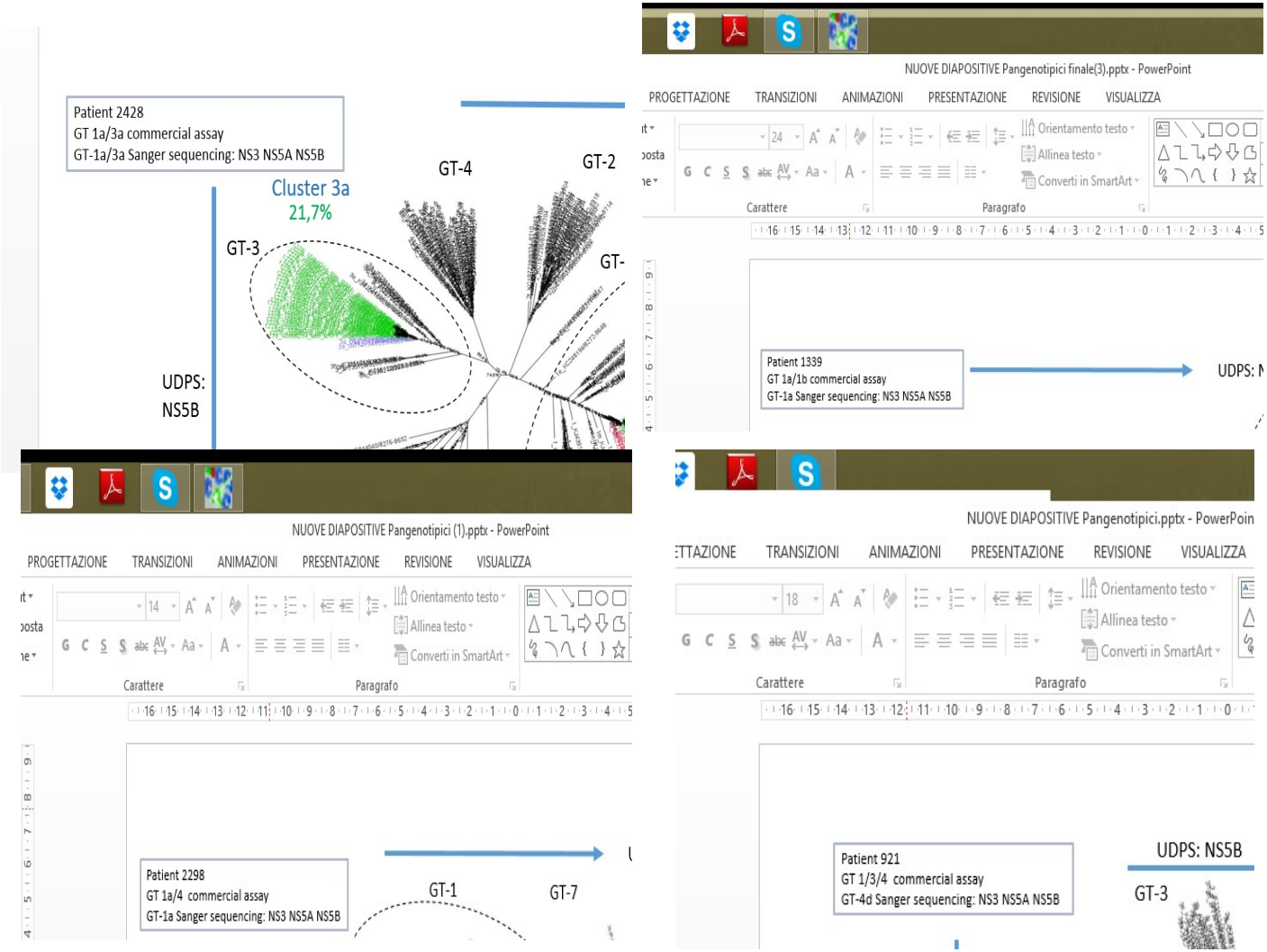
Susser S. et al., EASL 2016

Recombination breakpoint



Recombination of all 2k/1b variants at the same site within HCV *NS2* gene.

4 cases of patients infected by “mixed” HCV infection identified by UDPS



Aragri M et al., et al., 15th European Meeting on HIV & Hepatitis 2017

CONCLUSIONS (I)

- Viruses with high propensity to variability require drug combinations to be controlled

- There is a wide number of reasons for studying viral evolution in medical virology, in the perspective of the development of:
  - Reliable diagnostic tools
  - Clinically active antivirals
  - Broadly effective vaccines

CONCLUSIONS (II)

- It is important that as far as possible all new drugs and targets should combine a propensity for a low frequency of resistance selection, high fitness costs associated with resistance and low probability of fitness compensation

Grazie per l’attenzione!