JORNADAS DE LA SOCIETAT CATALANA DE MALALTIES INFECCIOSES I MICROBIOLOGIA CLÍNICA

Aplicabilitat clínica dels estudis de resistències del VIH

Lleida, 4 y 5 de Noviembre del 2011

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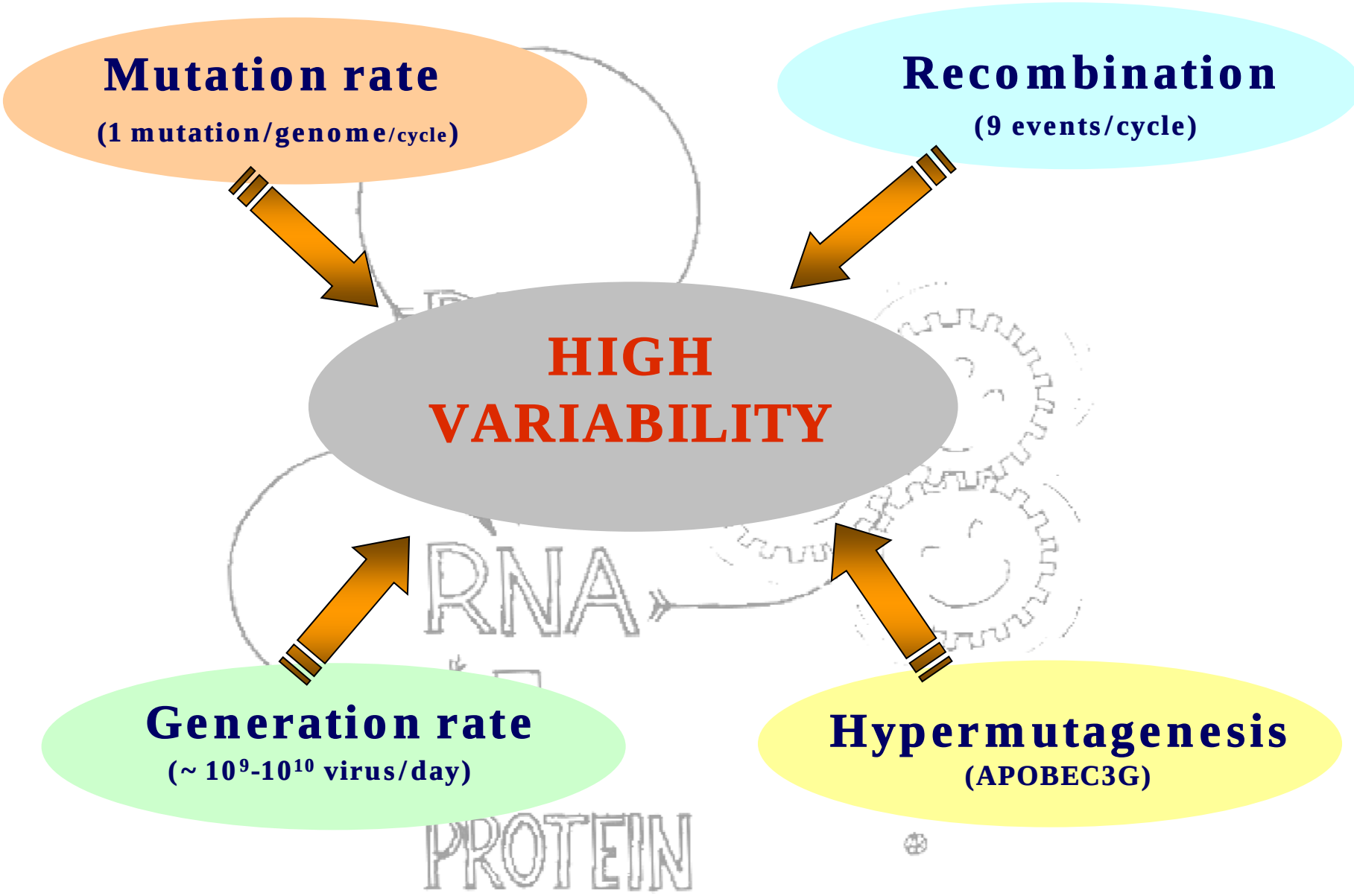
Resistance: General concept

Overall of mechanism that a microorganism can develop to evade the action of the drug.

Changes in targets not compromising target function but impeding drug linking

Key of Adaptation – Genetic Evolution



Factores relacionados con el virus

Factores relacionados con el huesped y el tratamiento.

Resistencias:
- **Naturales**
- **Transmitidas**

- **Problemas de adherencia**
- **Farmacocinética/fármacodinámica**
- **Falta de potencia**
- **Otros???**

Replicación Vírica Persistente

Desarrollo de Resistencias Farmacológicas

Fracaso Terapéutico

ORIGINAL ARTICLE

Original Article REPORT

◄ Previous Volume 328:1163-1165 April 22, 1993 Number 16 Next ►

Primary Infection with Zidovudine-Resistant Human Immunodeficiency Virus Type 1

Alejo Erice, Douglas L. Mayers, David G. Strike, Kim J. Sannerud, Francine E. McCutchan, Keith Henry, and Henry H. Balfour

Strains of human immunodeficiency virus type 1 (HIV-1) with reduced sensitivity to zidovudine have been isolated from patients treated with this drug for six months or more[1]. Resistance to zidovudine is associated with late-stage disease, low CD4 lymphocyte counts, longer antiretroviral therapy, and specific mutations in the reverse transcriptase gene of HIV-1[2,3]. The clinical importance of infections with resistant HIV-1 isolates is not well understood. We describe a patient with symptomatic HIV-1 infection who had primary infection with a virus resistant to zidovudine, according to both phenotypic and genotypic analyses.

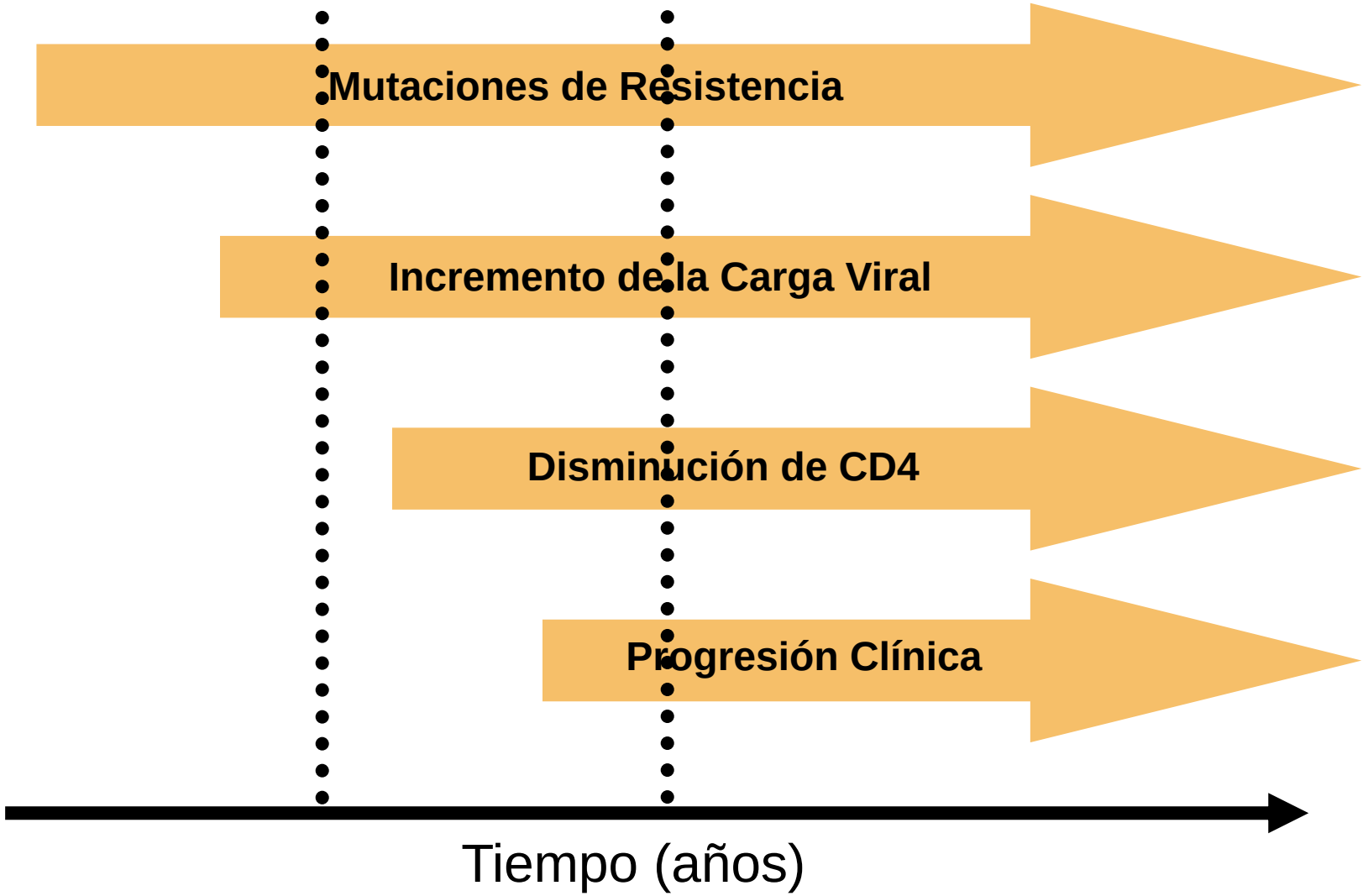
HIV-1 Isolate Obtained	IC_{50}*	Reverse Transcriptase Genotype†					
	μmol of zidovudine/liter	41	67	70	74	215	219
Before therapy	0.86	Met	Asp	Lys	Leu	*Tyr/Phe*	Lys
After therapy	2.90	Met	Asp	Lys	Leu	*Tyr/Phe*	Lys

*Denotes 50 percent inhibitory concentration.

†Numbers denote the positions on the reverse transcriptase gene encoding the amino acids shown. Mutations are indicated in italics.

Table 2. Phenotypic and Genotypic Characteristics of HIV-1 Isolates Obtained from the Patient before and after Zidovudine Therapy.

Impacto retardado de las resistencias en la progresión clínica



Multiple drug class-wide resistance associated with poorer survival after treatment failure in a cohort of HIV-infected patients

Mauro Zaccarelli[a], Valerio Tozzi[a], Patrizia Lorenzini[a], Maria P. Trotta[a], Federica Forbici[b], Ubaldo Visco-Comandini[a], Caterina Gori[b], Pasquale Narciso[a], Carlo F. Perno[b] and Andrea Antinori[a] for the Collaborative Group for Clinical Use of HIV Genotype Resistance Test (GRT) at National Institute for Infectious Diseases 'Lazzaro Spallanzani'

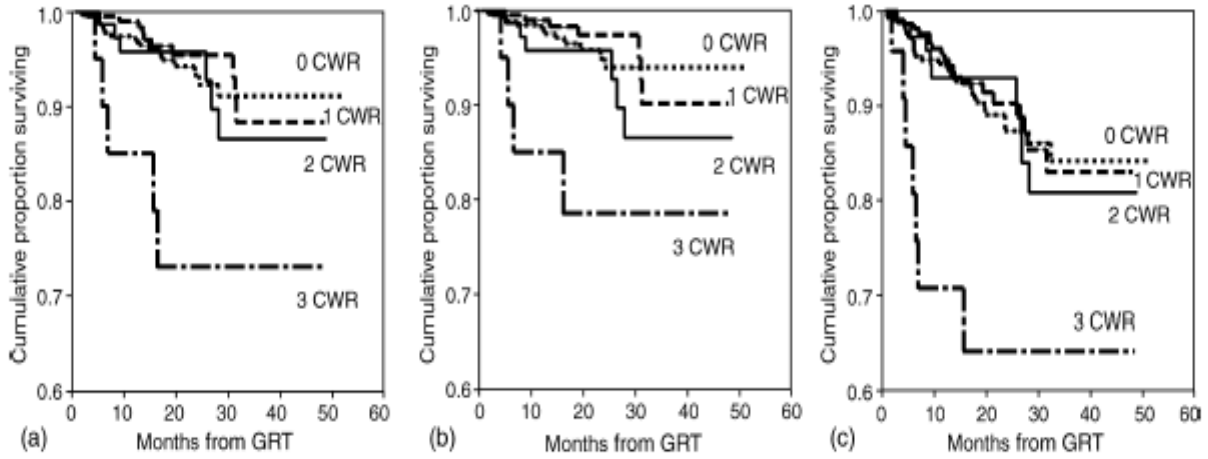


Fig. 3. Kaplan–Meier cumulative probability of surviving (a), an AIDS-related death (b) or surviving or remaining free from AIDS events (c) in patients with none, one, two or three three CWR. (a,b) At times 0, 1, 2 or 3 years, the numbers of patients at risk with 0 CWR were 306, 226, 100, 31, respectively; with 1 CWR were 215, 168, 69, 25, respectively; with 2 CWR were 78, 62, 38, 17, respectively; and with three CWR were 24, 16, 12, 7, respectively. (c) At times 0, 1, 2 or 3 years, the numbers of patients at risk with 0 CWR were 306, 218, 95, 28, respectively; with 1 CWR were 215, 163, 67, 24, respectively; with 2 CWR were 78, 60, 36, 15, respectively; and with three CWR 24, 13, 9, 5, respectively. CWR, class-wide resistance; GRT, genotypic resistance testing.

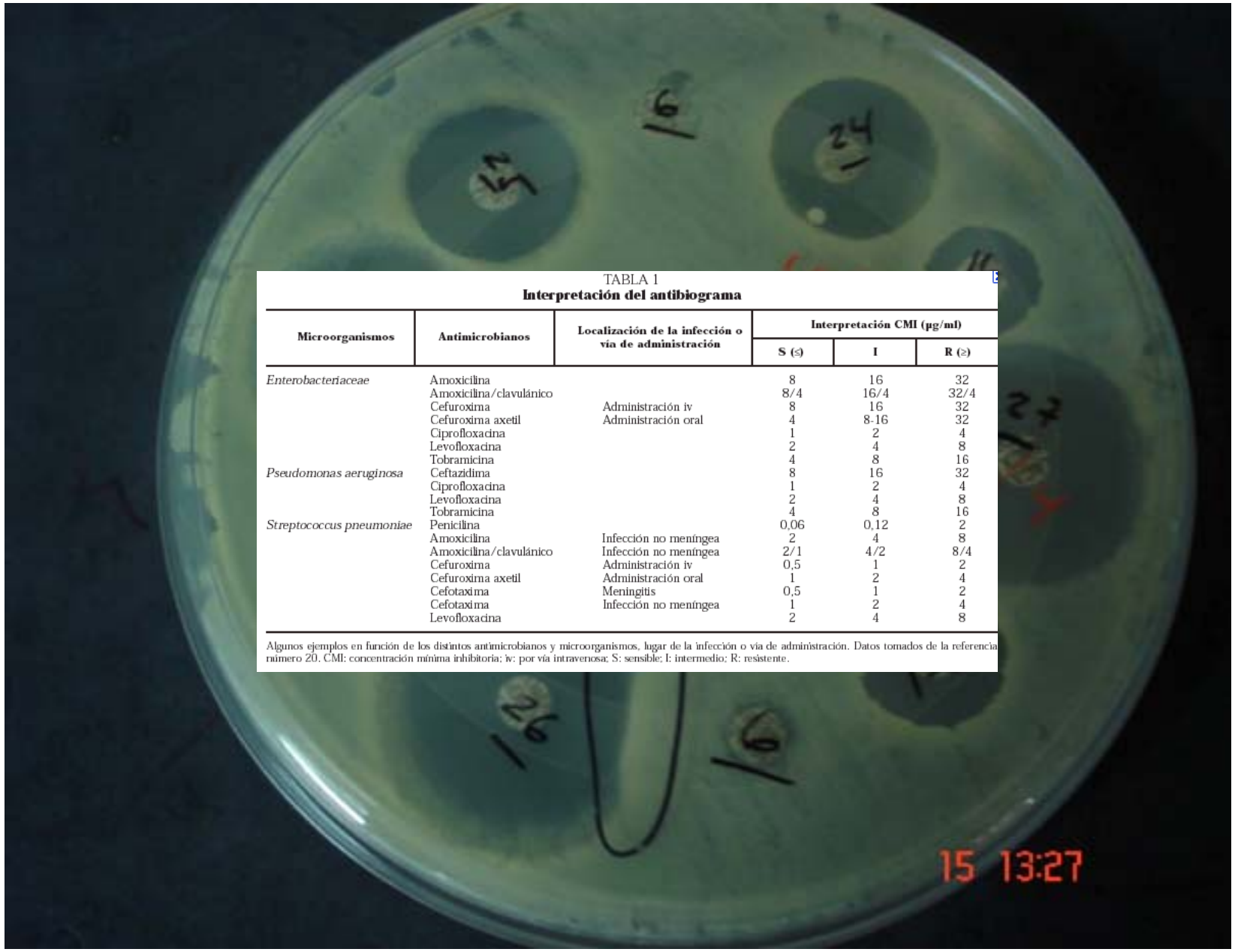


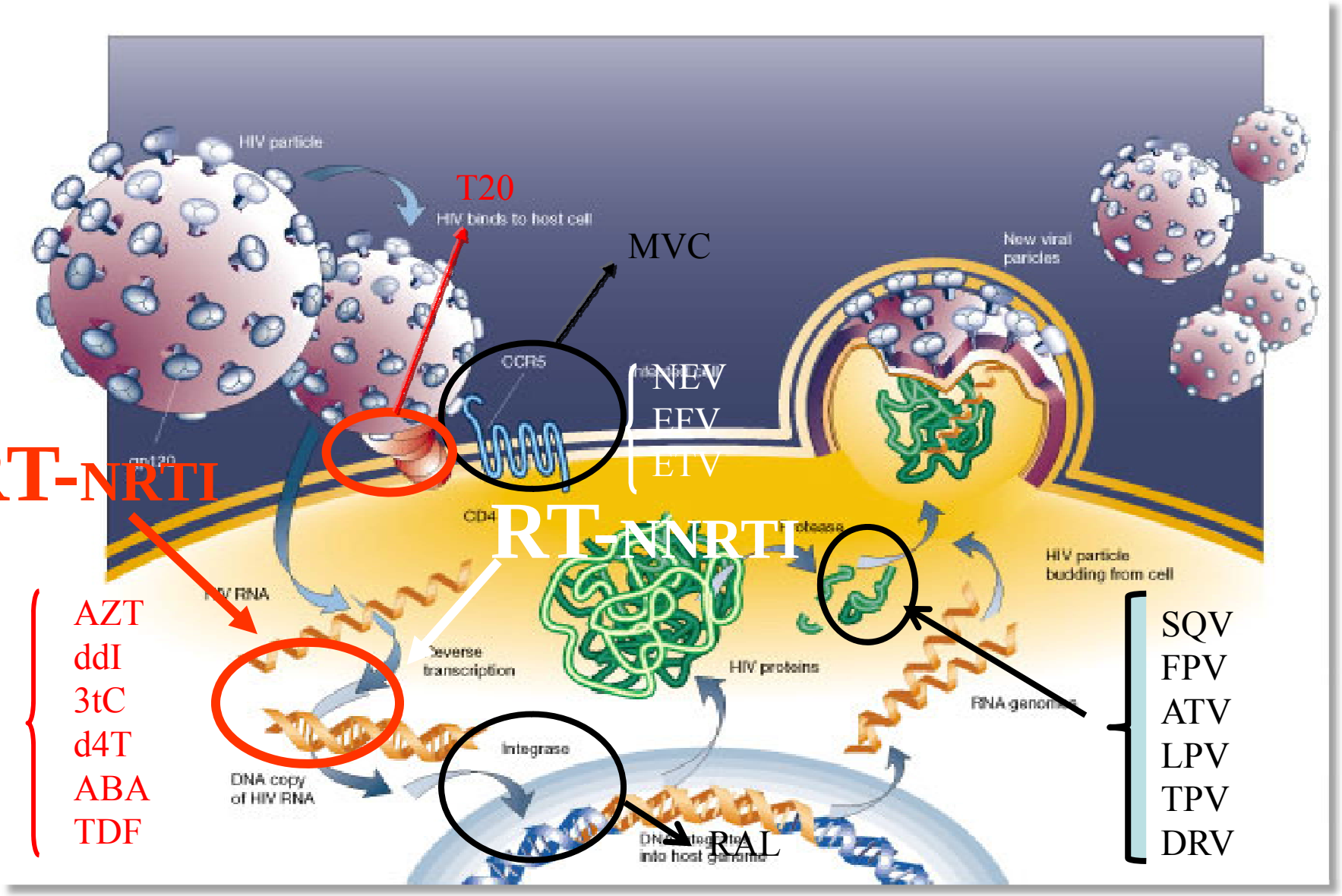
TABLA 1

Interpretación del antibiograma

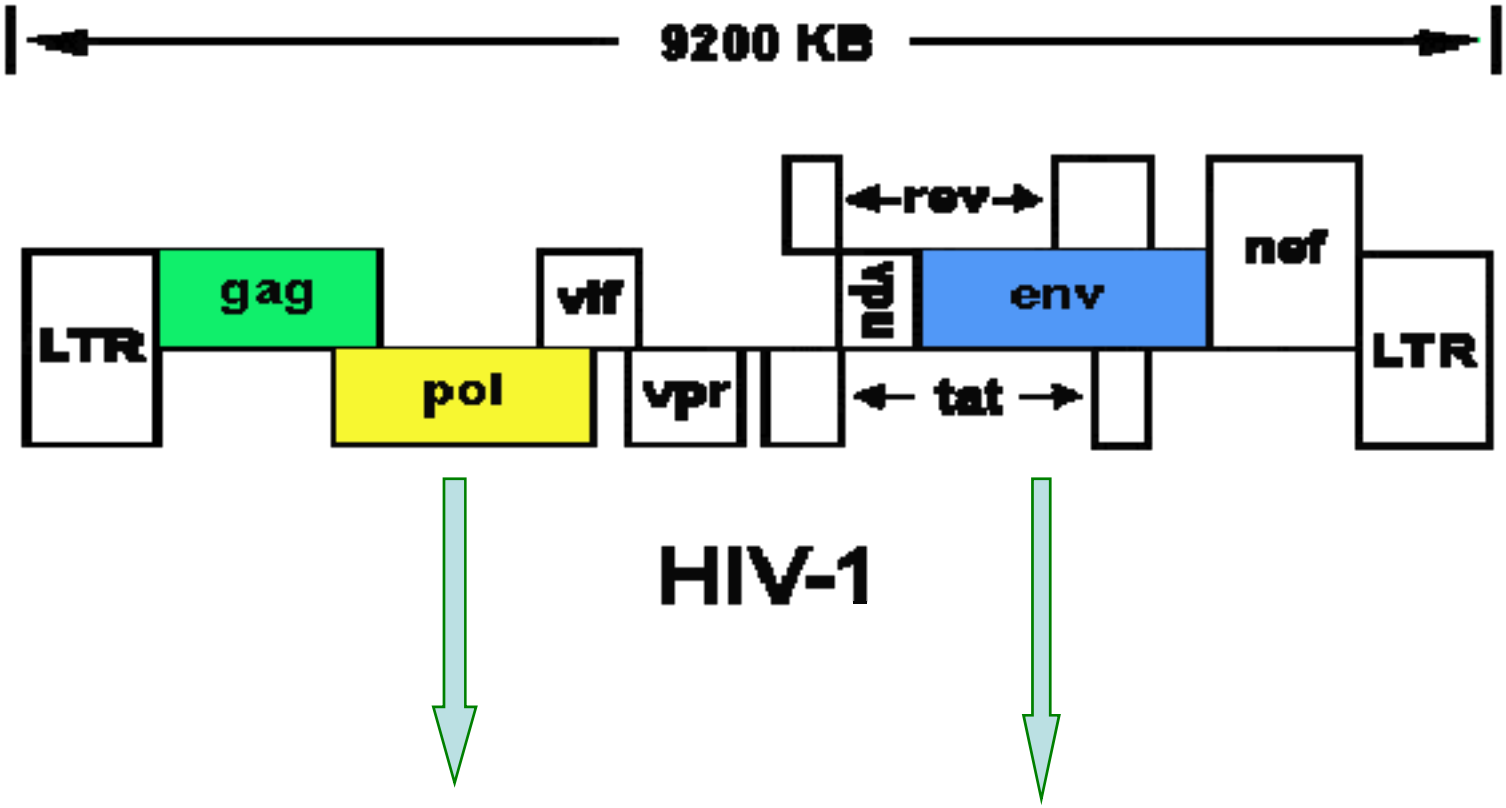
Microorganismos	Antimicrobianos	Localización de la infección o vía de administración	Interpretación CMI (µg/ml)		
			S (≤)	I	R (≥)
Enterobacteriaceae	Amoxicilina		8	16	32
	Amoxicilina/clavulánico		8/4	16/4	32/4
	Cefuroxima	Administración iv	8	16	32
	Cefuroxima axetil	Administración oral	4	8-16	32
	Ciprofloxacina		1	2	4
	Levofloxacina		2	4	8
	Tobramicina		4	8	16
Pseudomonas aeruginosa	Ceftazidima		8	16	32
	Ciprofloxacina		1	2	4
	Levofloxacina		2	4	8
	Tobramicina		4	8	16
Streptococcus pneumoniae	Penicilina		0,06	0,12	2
	Amoxicilina	Infección no meníngea	2	4	8
	Amoxicilina/clavulánico	Infección no meníngea	2/1	4/2	8/4
	Cefuroxima	Administración iv	0,5	1	2
	Cefuroxima axetil	Administración oral	1	2	4
	Cefotaxima	Meningitis	0,5	1	2
	Cefotaxima	Infección no meníngea	1	2	4
	Levofloxacina		2	4	8

Algunos ejemplos en función de los distintos antimicrobianos y microorganismos, lugar de la infección o vía de administración. Datos tomados de la referencia número 20. CMI: concentración mínima inhibitoria; iv: por vía intravenosa; S: sensible; I: intermedio; R: resistente.

Life Cycle VIH-1



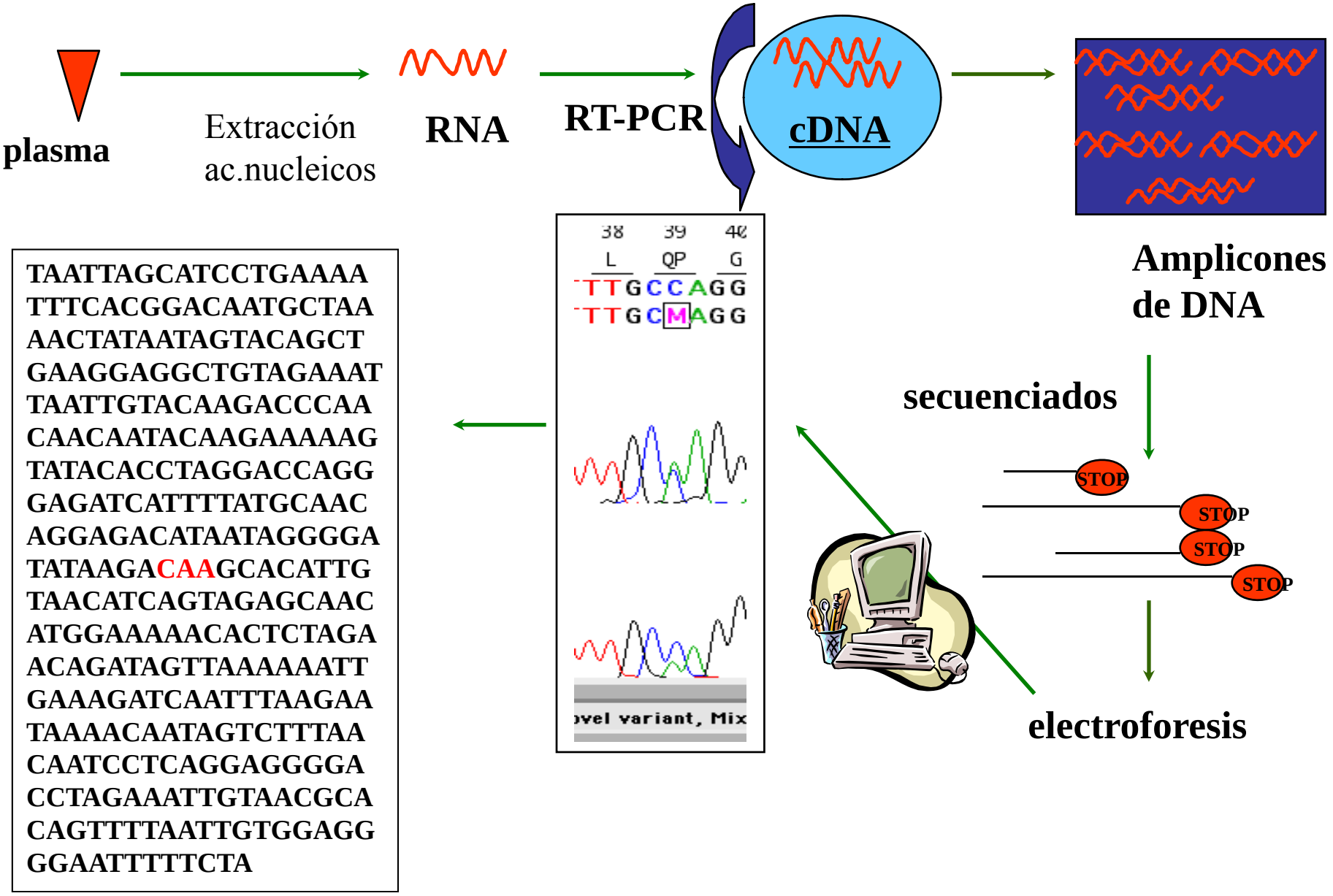
Genoma del VIH



RT: NRTI / NNRTI
PRT: IPs
Integrasa: Inh. Integrasa

Gp41-120: T-20
Gp 120 : Inh CCR5

Métodos Genotípicos. Secuenciación



Nomenclatura de las Mutaciones

Codon #184

AAT- CTG …. AAC - AUG

aa_1—aa_2—…—aa_{183}—aa_{184}

Si suponemos que la base G muta a T:

AAT- CTG …. AAC - GUG

aa_1—aa_2—…—aa_{183}—aa_{184}

Nomenclatura Mutación:
M184V

AUG = Methionine (M) GUG = Valine (V)

Code	Amino acids
A	alanine
C	cysteine
D	aspartate
E	glutamate
F	phenylalanine
G	glycine
H	histidine
I	isoleucine
K	lysine
L	leucine
M	methionine
N	asparagine
P	proline
Q	glutamine
R	arginine
S	serine
T	threonine
V	valine
W	trytophan
Y	tyrosine

TAATTAGCATCCTGAAAA
TTTCACGGACAATGCTAA
AACTATAATAGTACAGCT
GAAGGAGGCTGTAGAAAT
TAATTGTACAAGACCCAA
CAACAATACAAGAAAAAG
TATACACCTAGGACCAGG
GAGATCATTTATGCAAC
AGGAGACATAATAGGGGA
TATAAGACAAGCACATTG
TAACATCAGTAGAGCAAC
ATGGAAAAUGACACTCTA
GAACAGATAGTTAAAAAA
TTGAAAGATCAATTTAAG
AATAAAACAATAGTCTTTA
ACAATCCTCAGCAGGGG
ACCTAGAAATTGTAACGC
ACAGTTTTAATTGTGGAG
GGGAATTTTTCTA

Nº Identificación Muestra: 4002
Fecha: 1/11/2000
CV: 8000 cp/ml
Hospital: HCP
Médico Solicitante: Dr Blanco

Sistema de Genotipado: PE Biosystems
Cepa de Referencia: pNL-4 Fecha del Informe: 12/11/00

Mutaciones para los Inhibidores Nucleósidos de la Retrotranscriptasa: M184V R211K

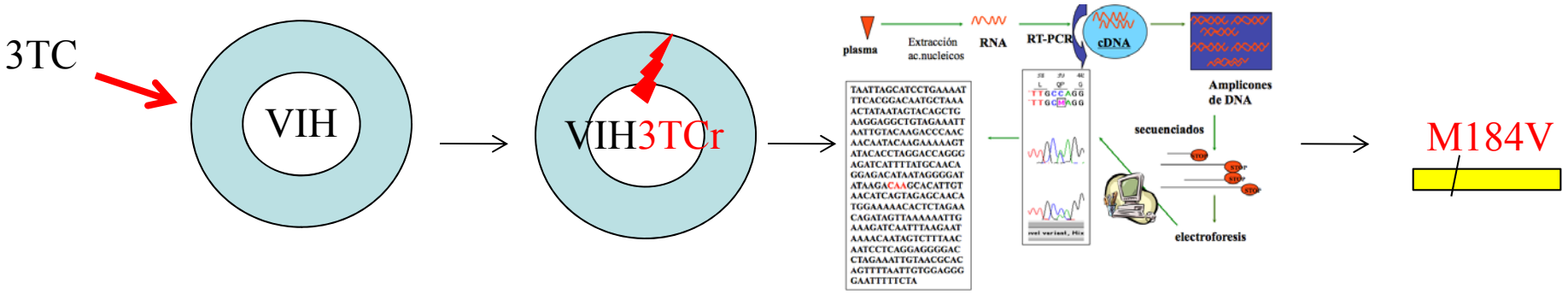
Zidovudina	No evidencia de Resistencia
Didanosina	Resistencia parcial
Zalcitabina	Resistencia parcial
Lamivudina	Resistencia
Estavudina	No evidencia de Resistencia
Abacavir	Resistencia parcial
Adefovir	No evidencia de Resistencia

Mutaciones para los Inhibidores No Nucleósidos de la Retrotranscriptasa: K103N

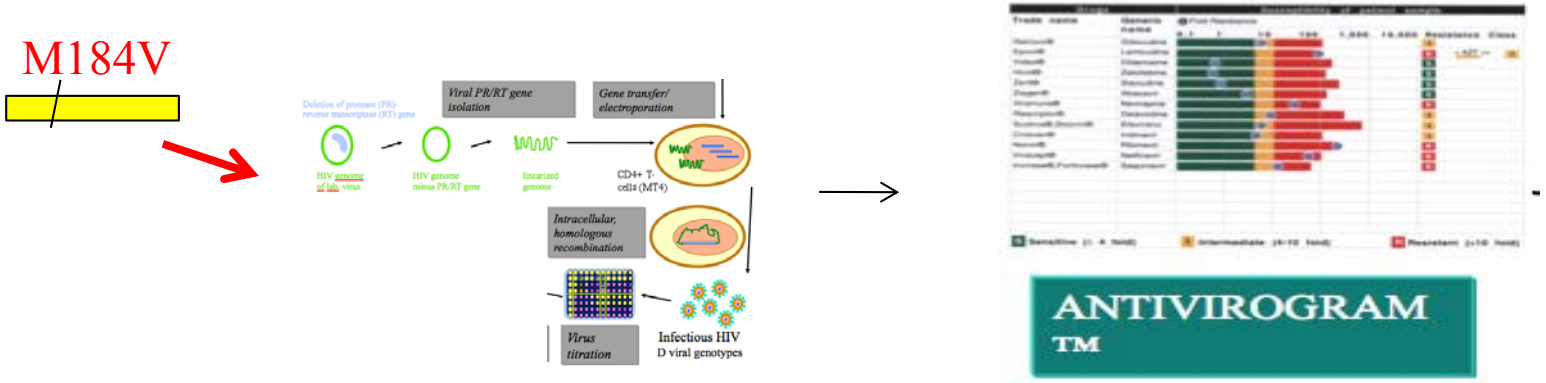
Nevirapina	Resistencia
Delavirdina	Resistencia
Efavirenz	Resistencia

¿Cómo sabemos que una mutación confiere resistencia a un fármaco?

Primer paso : Forzar la selección PASES IN VITRO



Segundo paso : Confirmación MUTAGENESIS DIRIGIDA

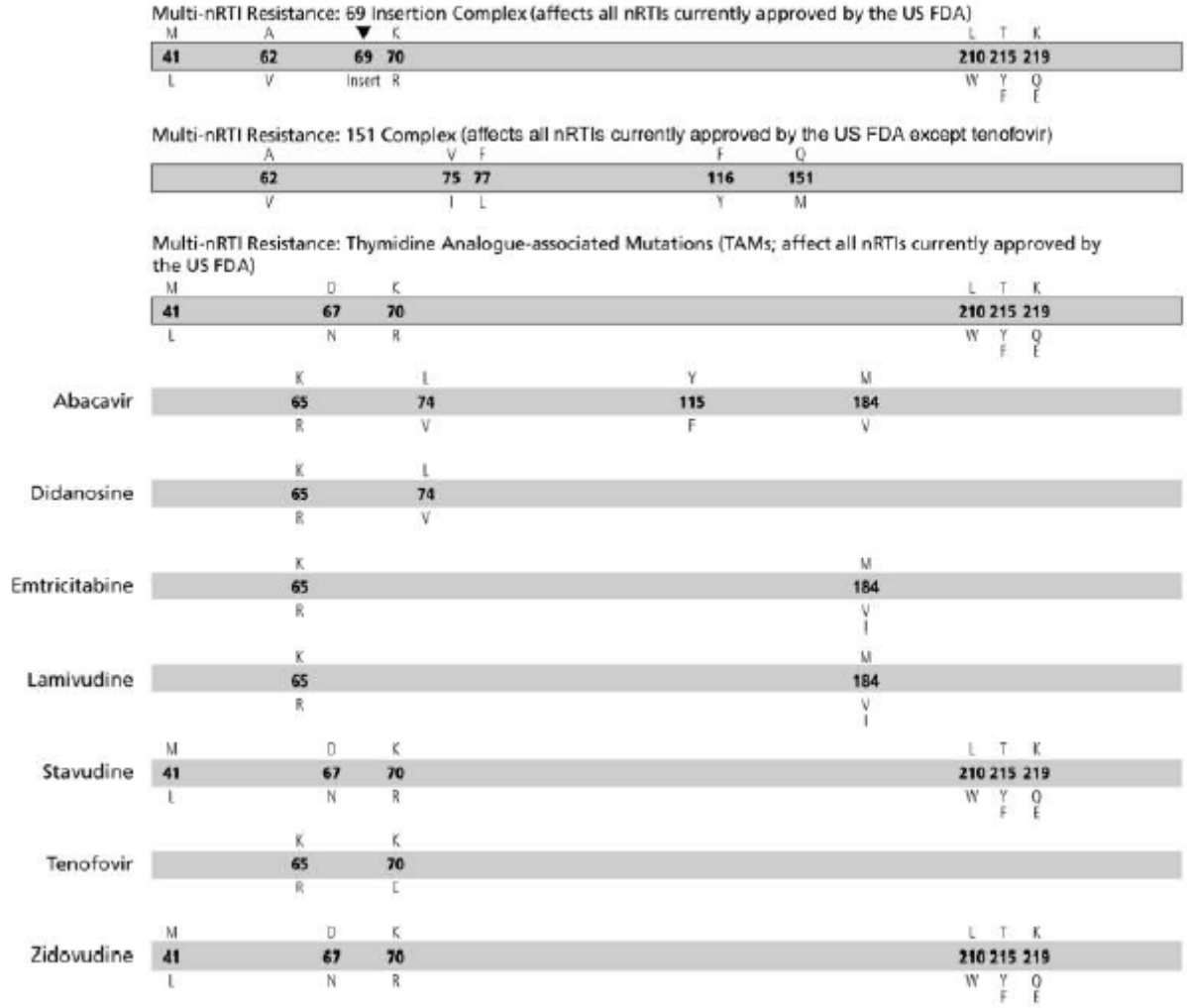


Tercer paso : ensayos clinicos (IIA,B; III y IV)

Antiretroviral Drug Resistance Testing in Adult HIV-1 Infection: 2008 Recommendations of an International AIDS Society–USA Panel

MUTATIONS IN THE REVERSE TRANSCRIPTASE GENE ASSOCIATED WITH RESISTANCE TO REVERSE TRANSCRIPTASE INHIBITORS

Nucleoside and Nucleotide Reverse Transcriptase Inhibitors (nRTIs)



STANFORD UNIVERSITY

HIV DRUG RESISTANCE DATABASE

A curated public database designed to represent, store, and analyze the divergent forms of data underlying HIV drug resistance.

Drug Resistance Interpretation: PR

PI Major Resistance Mutations:	M46I, I54A, V82A, L90M
PI Minor Resistance Mutations:	L10V, L33F, K43T, F53Y, A71V, T74P
Other Mutations:	I13V, L24F, E34Q, N37E, D60E, I62V, L63P, I64M, V77I, I93L

Protease Inhibitors

atazanavir/r (ATV/r)	High-level resistance
darunavir/r (DRV/r)	Susceptible
fosamprenavir/r (FPV/r)	High-level resistance
indinavir/r (IDV/r)	High-level resistance
lopinavir/r (LPV/r)	High-level resistance
nelfinavir (NFV)	High-level resistance
saquinavir/r (SQV/r)	High-level resistance
tipranavir/r (TPV/r)	Intermediate resistance

PR Comments

PIMajor

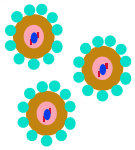
- M46I/L decreases susceptibility to IDV/r, NFV, FPV/r, LPV/r, and ATV/r when present with other mutations.
- I54T/A/S are PI-related mutations that appear to be associated with decreased susceptibility to each of the PIs. But their effects have not been as well studied as I54V, I54M, or I54L.
- V82A reduces susceptibility to IDV/r and LPV/r. With other mutations it is associated with reduced susceptibility to NFV, ATV/r, SQV/r, and FPV/r.
- L90M causes resistance to NFV, SQV/r, ATV/r, and IDV/r. When present with other mutations it also decreases the activity of FPV/r and LPV/r.

GENOTYPE-PHENOTYPE CORRELATIONS REFERENCES

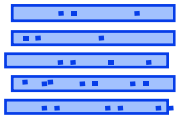
MARVEL (Mutation ARV Evidence Listing) » Go To Program

Métodos Fenotípicos. VIRCO

PATIENT



extraction → PLASMA → *RT* → total RNA → *PCR* → cDNA → PR/RT GENES

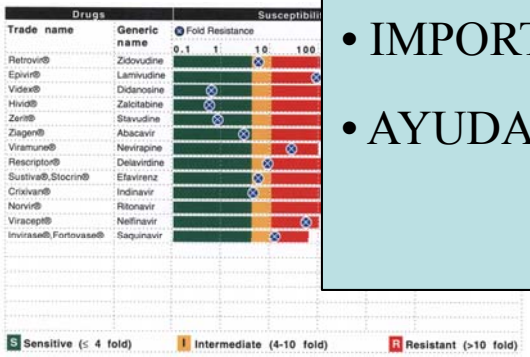


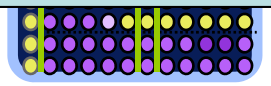
- CARO (más de 1000 $)
- COMPLEJO (tiempo de elaboración 2-4 semanas)
- POCOS LABORATORIOS (Virco, Virologic, VIRalliance)
- RESULTADOS NO SUPERIORES A METODOS GENOTIPICOS

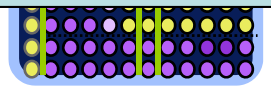
- IMPORTANTE EN FARMACOS NUEVOS
- AYUDA EN SECUENCIAS COMPLEJAS

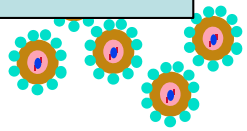
Deletion of protease (
reverse transcriptase (

HIV genome
of lab. virus









ANTIVIROGRAM
TM

Susceptibility Assay

Virus titration

Infectious HIV
D viral genotypes

Indicaciones del test de resistencias

	IAS-USA[1]	DHHS[2]	European[3]
Primary/acute	Recommend	Recommend	Recommend
Postexposure prophylaxis	—	—	Recommend
Chronic, Rx naive	Recommend*	Recommend	Strongly consider*
Failure	Recommend†	Recommend	Recommend
Pregnancy	Recommend	—	Recommend
Pediatric	—	—	Recommend

*If prevalence of drug resistance in untreated patients ≥ 5% (European: ≥ 10%); should be considered if the prevalence is unknown or if exposure to someone receiving antiretroviral drugs is likely.
†Resistance testing should also be considered if the HIV VL suppression achieved with a new antiretroviral regimen is not optimal.

Minority viral populations

	Protease							Reverse Transcriptase					
	10	36	46	48	54	63	82	41	67	69	184	210	215
NL4-3	Leu	Met	Met	Gly	Ile	Leu	Val	Met	Asp	Thr	Met	Leu	Thr
578 Bulk	–	–	–	–	–	–	–	–	–	–	–	–	–
578PRT1	–	–	–	–	–	–	–	–	–	–	–	–	–
578PRT2	–	–	–	–	–	–	–	–	–	–	–	–	–
578PRT3	–	–	–	–	–	–	–	–	–	–	–	–	–
578PRT8	–	–	–	–	–	–	–	–	–	–	–	–	–
578PRT9	–	–	–	–	–	–	–	–	–	–	–	–	–
578PRT10	–	–	–	–	–	–	–	–	–	–	–	–	–
578PRT11	–	–	–	–	–	–	–	–	–	–	–	–	–
578PRT12	–	–	–	–	–	–	–	–	–	–	–	–	–
578PRT13	–	–	–	–	–	–	–	–	–	–	–	–	–
578PRT14	–	–	–	–	–	–	–	–	–	–	–	–	–
578PRT16	–	–	–	–	–	–	–	Leu	–	–	–	–	–
578PRT4	–	–	–	–	nd	–	–	–	–	–	–	–	–
578PRT6	–	–	–	–	–	Pro	–	–	–	–	–	–	–
578PRT5	–	–	–	–	–	Pro	–	–	–	–	–	–	–
578PRT7	Ile	Ile	Ile	Val	Thr	Gln	Ala	Leu	Asn	Asp	Val	Trp	Tyr
578PRT15	Ile	Ile	Ile	Val	Thr	Gln	Ala	Leu	Asn	Asp	Val	Trp	Tyr

Martinez-Picado et al., J Clin Microbiol. 1999

Standard Sanger Sequencing Detects the Most Common Circulating HIV-1 Variants