Hepatitis B Drug Resistance: Navigating the Way Forward

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The paradigm of antiviral therapy is the suppression and maintenance of viraemia below the limit of detection

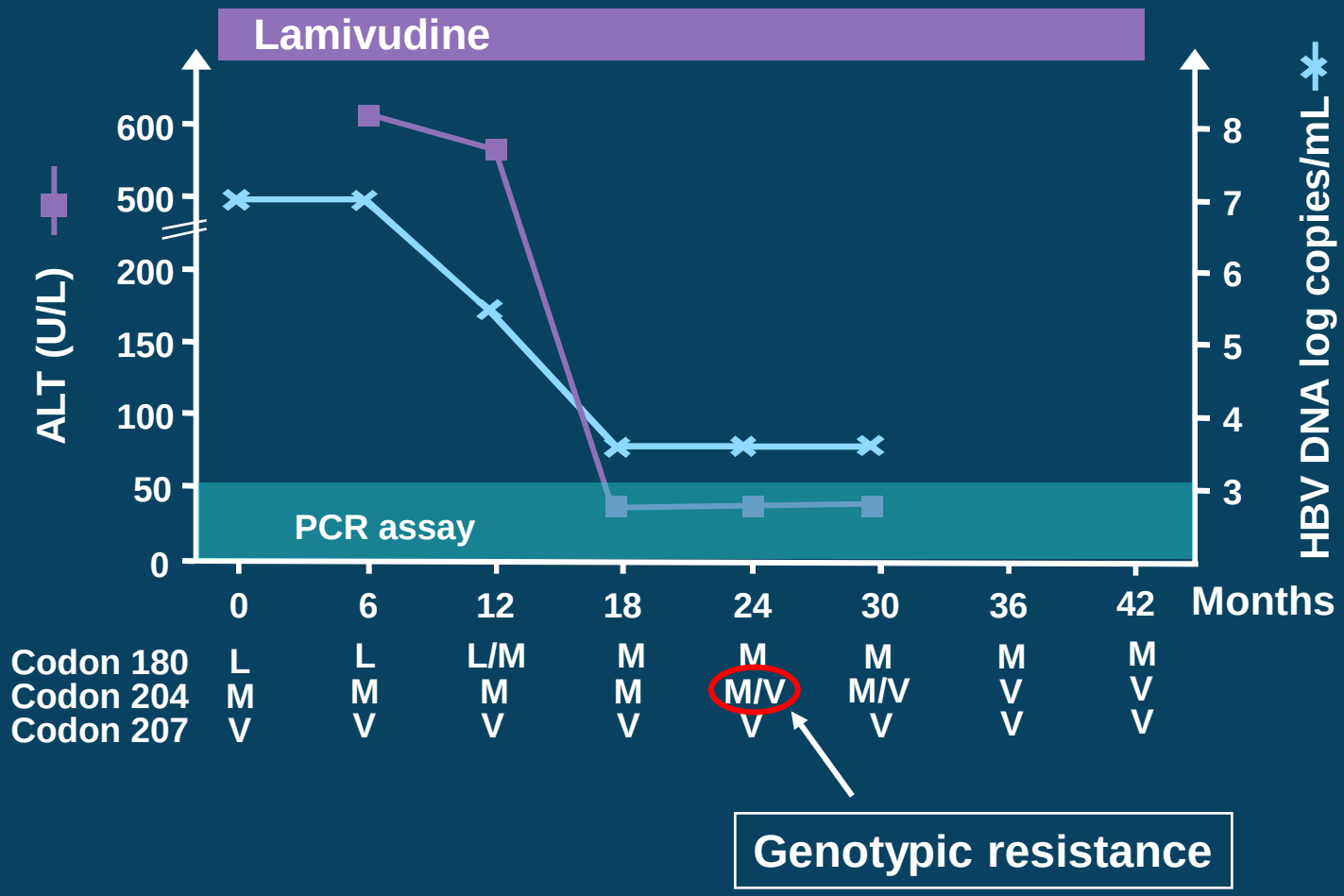
EASL Clinical Practice Guidelines Panel. J Hepatol. 2009;50:227-42.

Indications of Emergence of Drug-Resistant Virus

1. Increasing viral load (≥ 1.0 log IU/ml)
2. Identification of known genotypic markers of drug resistance within viral polymerase:
   * primary resistance mutations (rtM204I)
   * secondary resistance mutations (rtL180M with rtM204V)
   * compensatory mutations (rtV173L)
3. Increasing serum ALT levels
4. Clinical deterioration

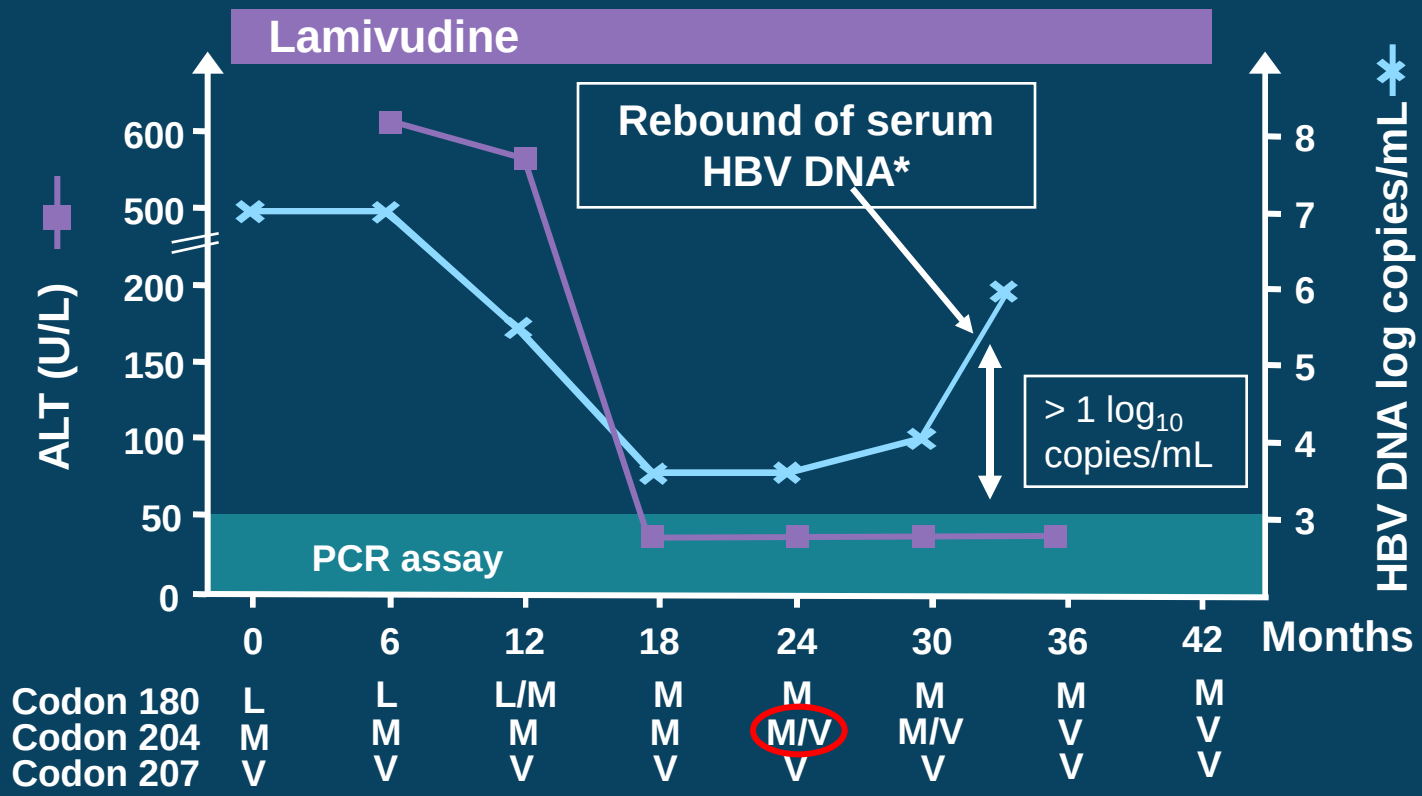
Ono-Nita S. et al 1999. J Clin Invest;103:1635; Ono S. et al 2001. J Clin Invest;107:449; Chin R. et al 2001. AAC;45:2495.

Dynamics of Resistance Emergence Genotypic Resistance



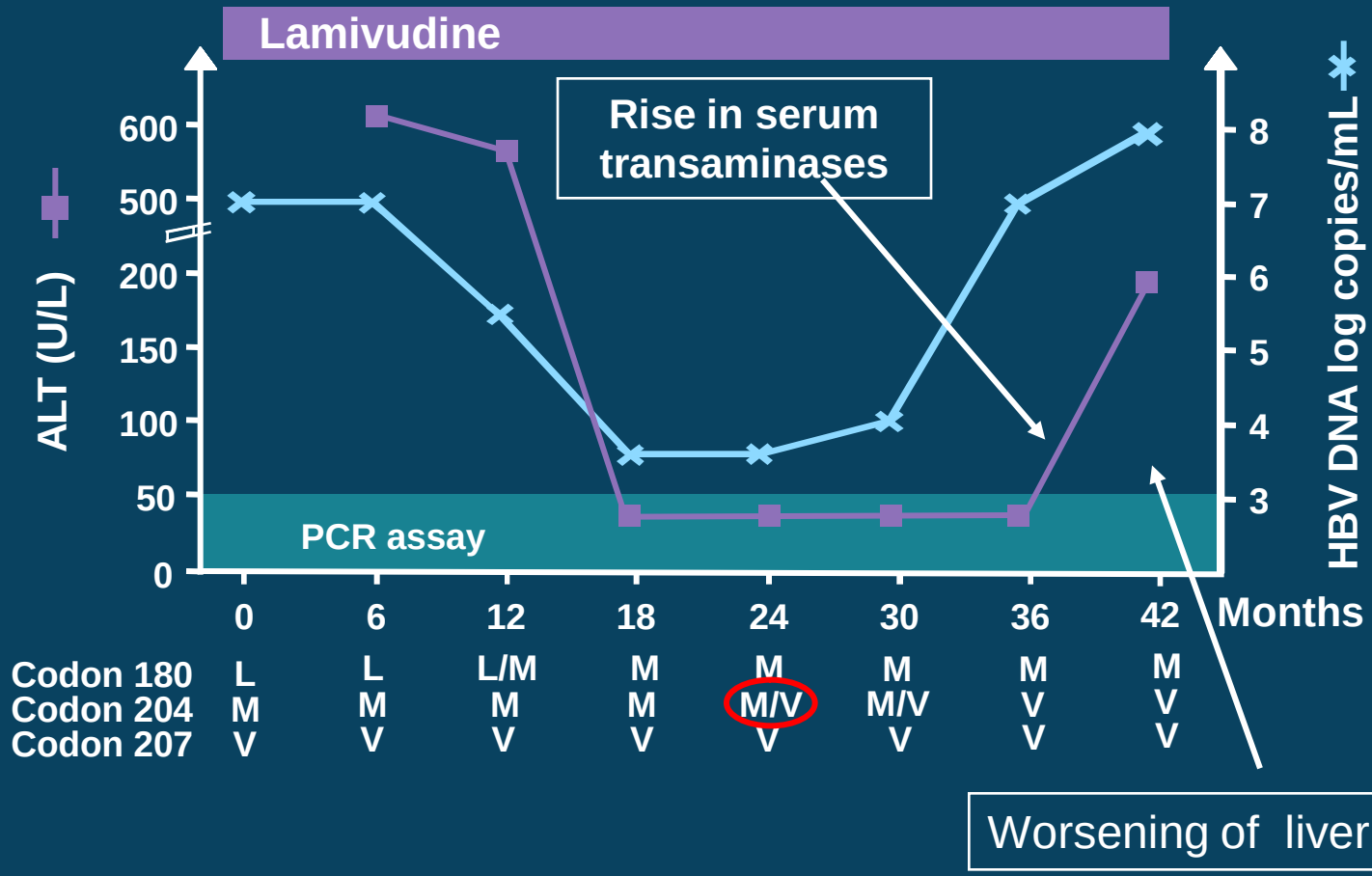
Si Ahmed et al. Hepatology 2000;32:1078–88

Dynamics of Resistance Emergence Virologic Breakthrough



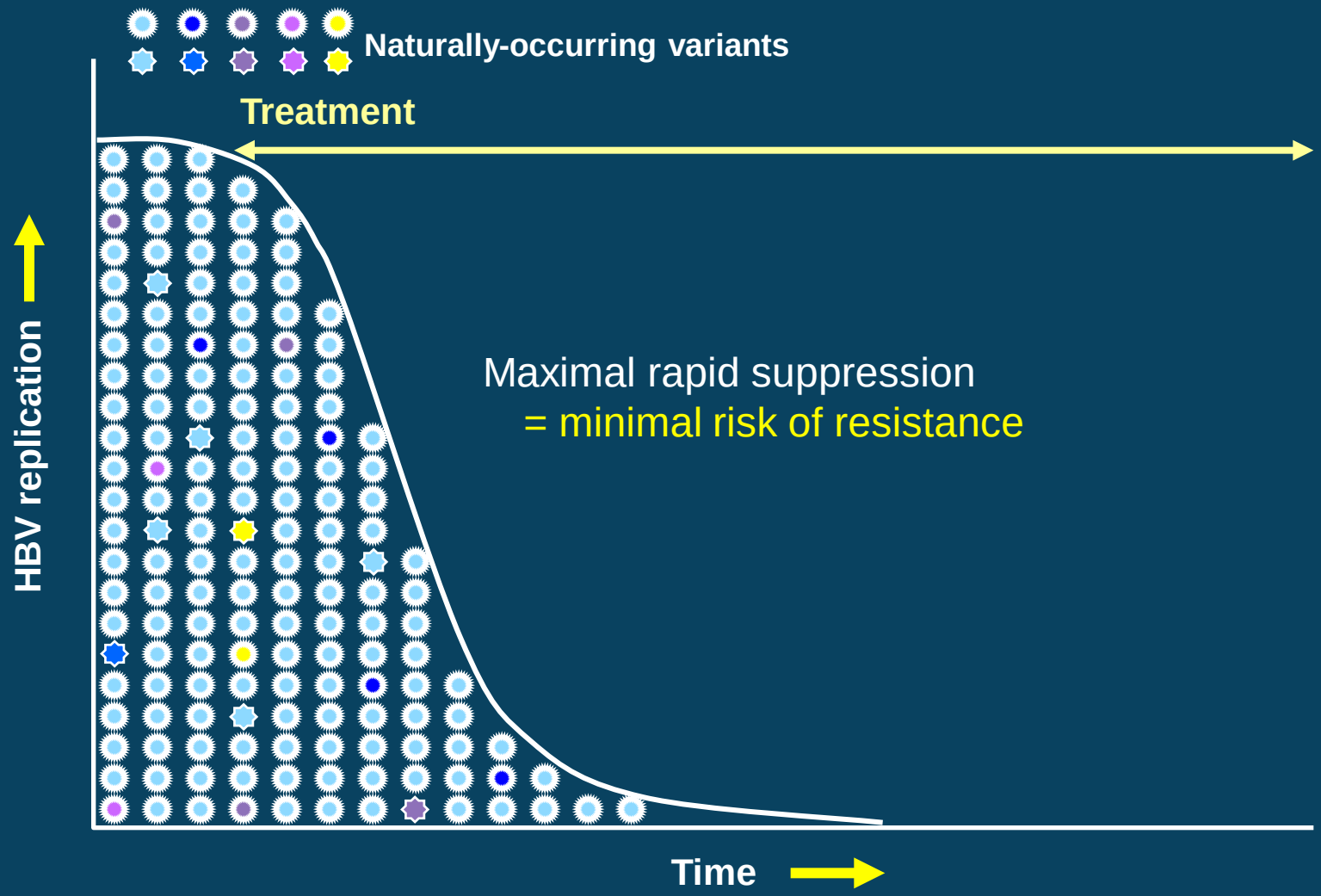
Si Ahmed et al. Hepatology 2000;32:1078–88

Dynamics of Resistance Emergence Clinical Breakthrough



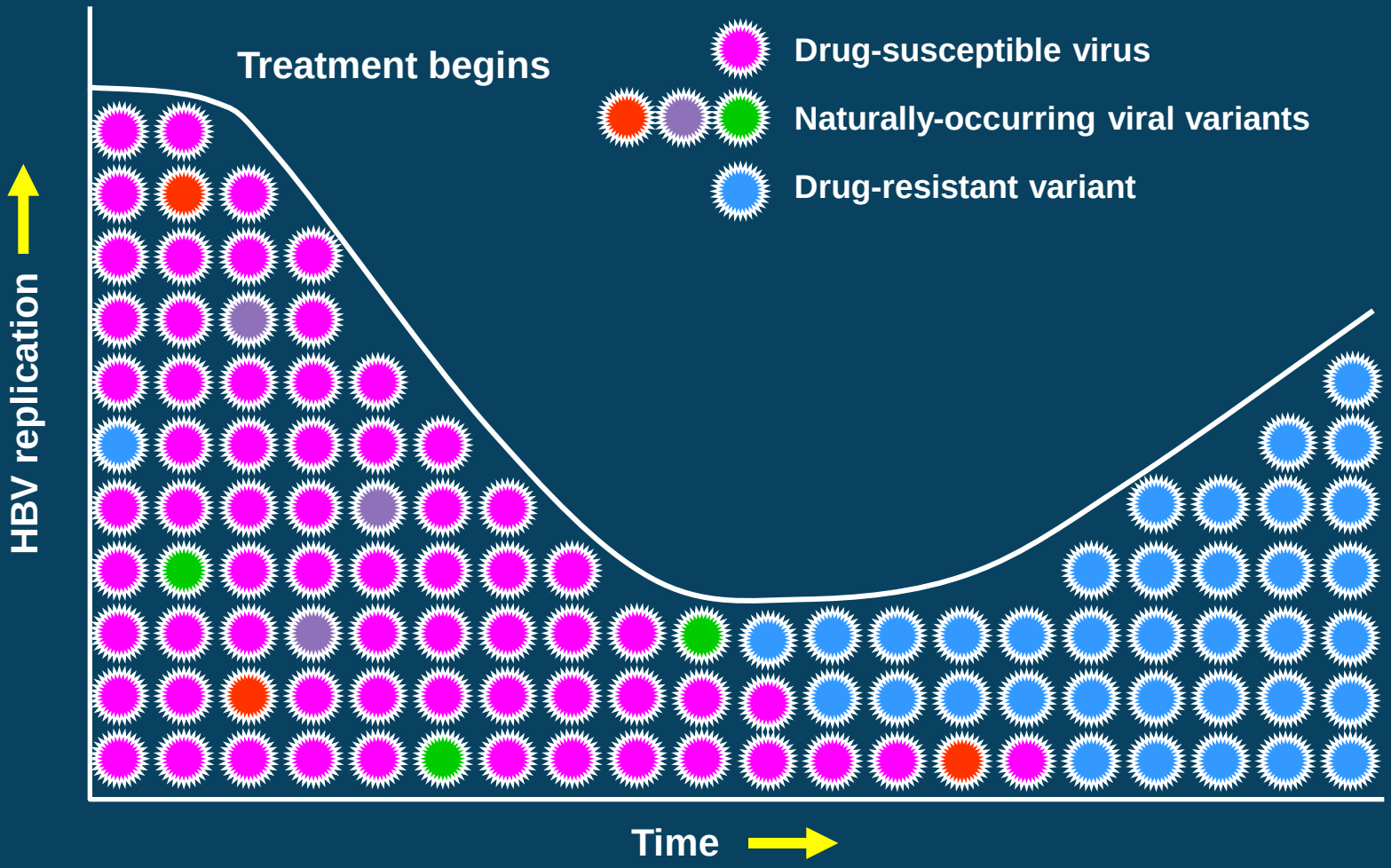
Si Ahmed et al. Hepatology 2000;32:1078–88

Drug Potency and Maximal Suppression of Viral Replication



Locarnini S, et al. Antivir Ther 2004;9:679–693.

Incomplete Suppression of Viral Replication Allows the Selection of Resistant Virus



1.Fung SK & Lok ASF. Antivir Ther 2004; 9:1013–1026 2. Locarnini S, et al. Antivir Ther 2004; 9:679–693.

Viral Replication and Mutational Frequency

- High virion production: $10^{12\text{-}13}$ virions per day
- Wild-type HBV Pol lacks proof-reading function
- High mutational rate:10^{-5} substitution/base/cycle
- $10^{10\text{-}11}$ point mutations produced per day
- All possible single base changes can be produced per day
- Single / double mutations pre-exist in HBV from patients prior to therapy: WHY MOST MONOTHERAPIES FAIL
- Triple / quadruple mutations require replication in the presence of selection pressure and rarely pre-exist: WHY COMBINATION TREATMENT WORKS

(Colgrone & Japour. 1999. AVR. 41:45)

Primary Resistance Substitutions

F__V__LLAQ__YMDD

L-Nucleoside Resistance

LMV	rtA181T/V	rtM204V/I	
L-dT	rtA181T/V	rtM204I	

Acyclic Phosphonate Resistance

ADV/TFV	rtA181T/V	rtN236T

D-Cyclopentane Resistance

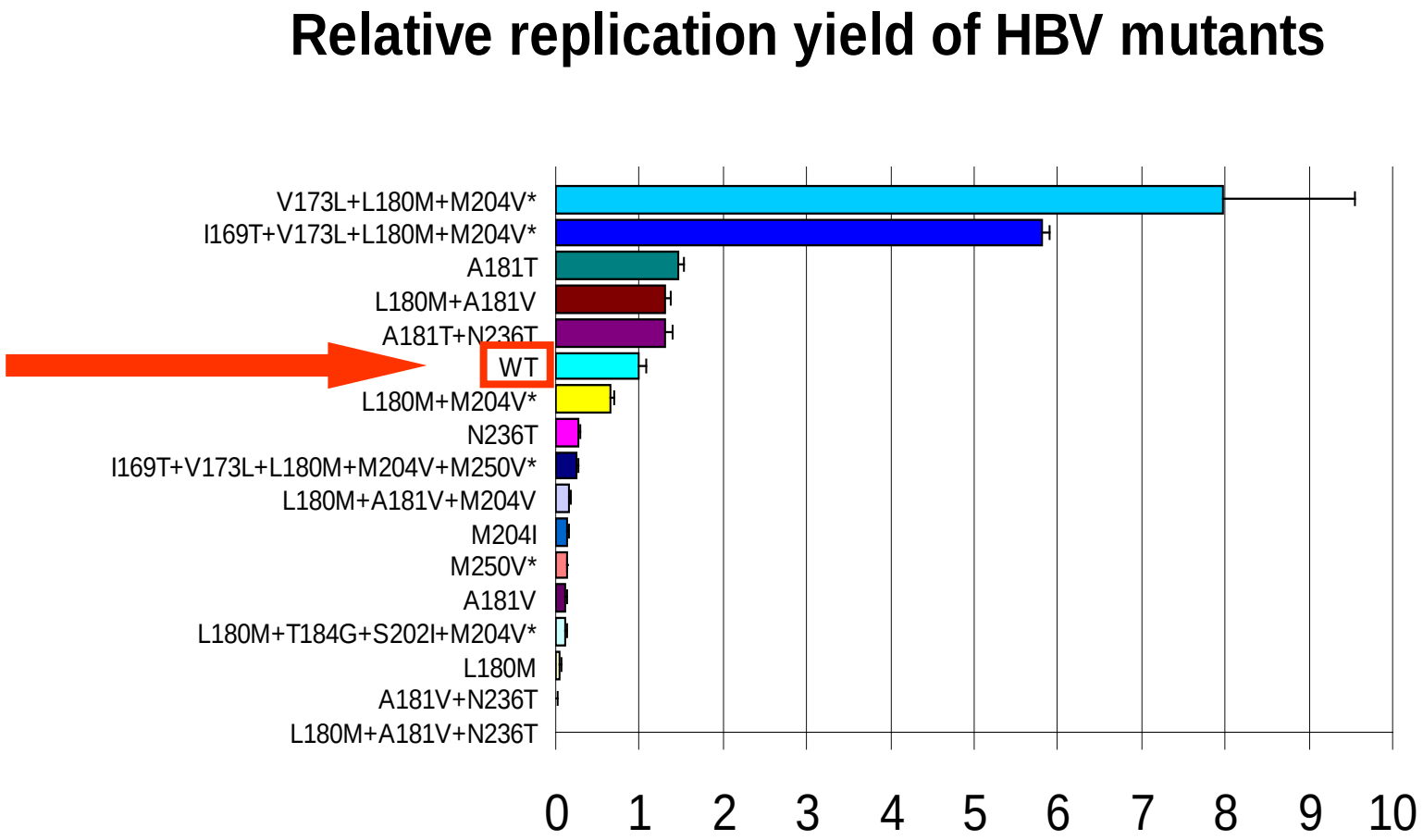
ETV	rtL180M plus	rtM204V/I		
	rtI169	rtT184	rtS202	rtM250

Pathways of Antiviral Resistance in Chronic Hepatitis B

Pathway	Mutation	Associated Resistance
L-nucleoside	rtM204V/I	Lamivudine Emtricitabine Telbivudine
Acyclic phosphonate	rtN236T	Adefovir Tenofovir
"Shared"	rtA181T/V	L- nucleosides (see above) Acyclic phosphonates (see above)
naïve entecavir resistance	rtL180M + rtM204V with one of rtT184, S202 or M250	Entecavir
Multi-drug resistance	Complex patterns e.g. rtA181T + rtN236T + rtM250L	Multi-drug

Yuen, L. et al 2007. Hepatology vol 46 (No.4, Suppl.1) 659A Abstract #949

Compensatory Substitutions

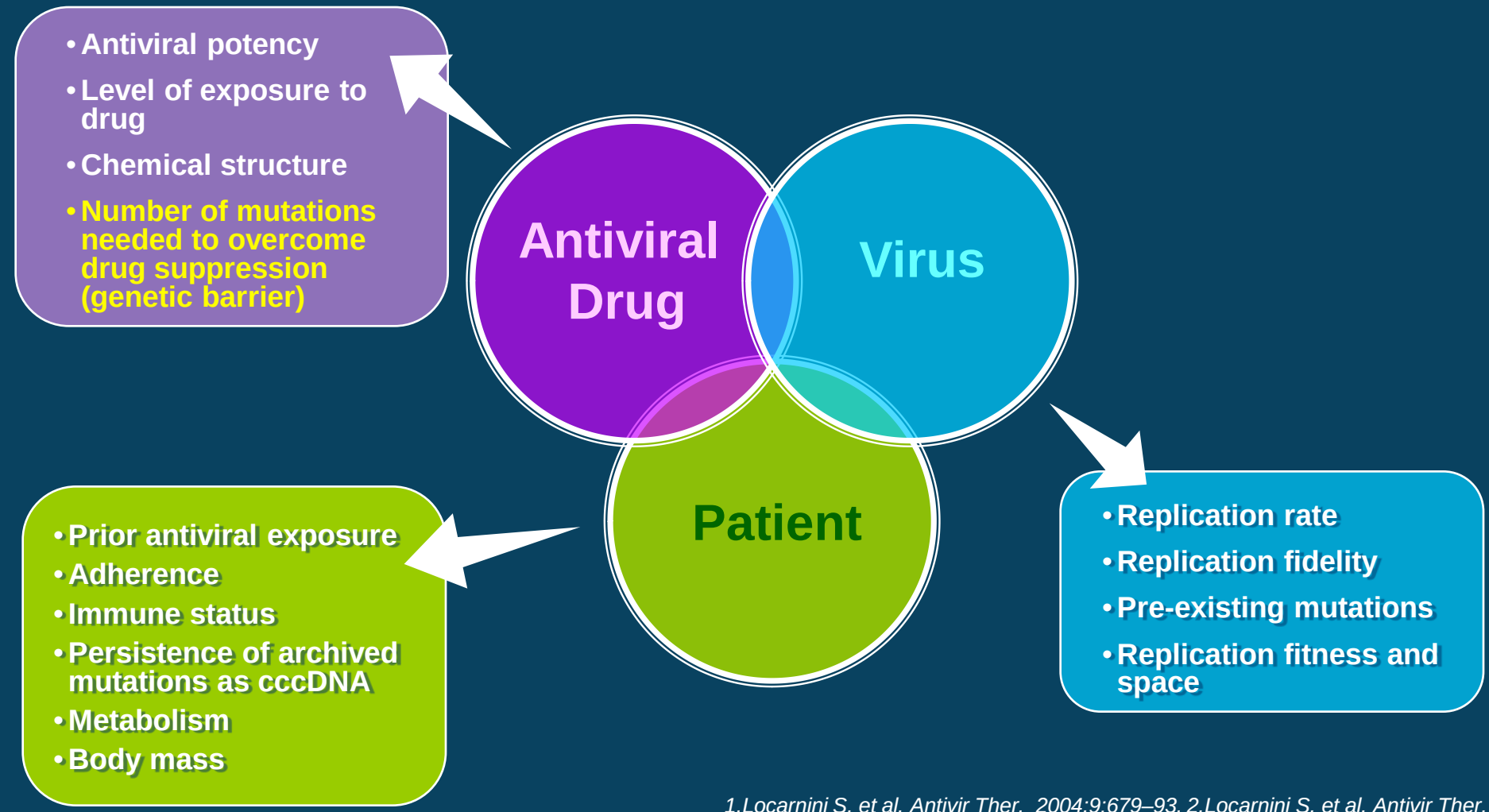


From: R. Edwards, T. Shaw, V. Sozzi & S. Locarnini. 2005. HepDART

Multi-Drug Resistance (MDR)

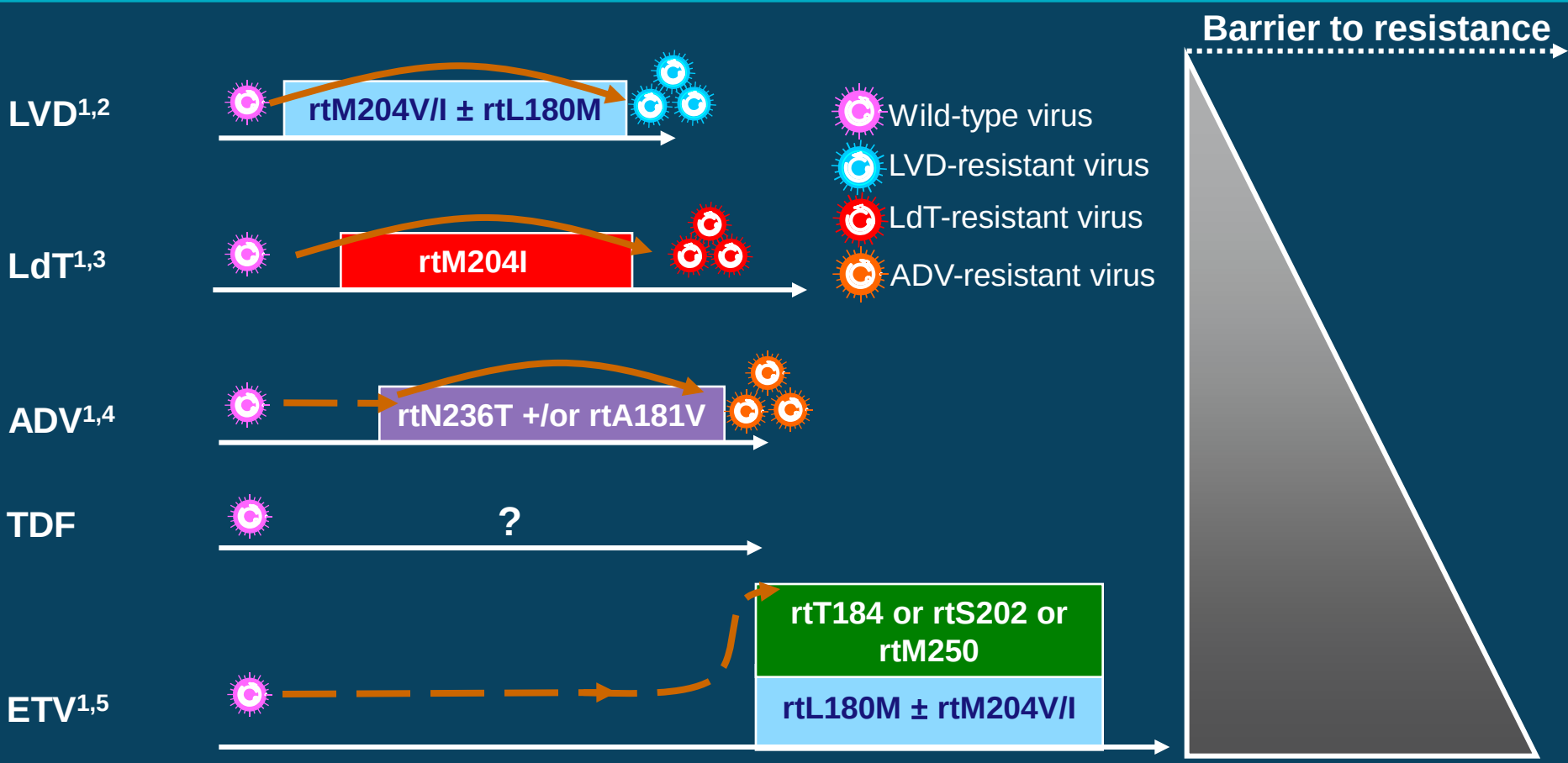
- Sequential addition of resistance mutations to the same viral genome
- **Promoted with sequential monotherapy**, especially by using drugs with similar (structural) characteristics
- **Role of compensatory mutations** virus replication competence (fitness)
- **Need for drug-resistance testing** (Pol sequencing) to determine and monitor therapy:
  - rtA181T (“Shared” Pathway)
  - rtA181T+rtN236T
  - rtA181T+rtI233V+rtN236T+rtM250L

Factors Associated with the Emergence of Resistance to CHB Therapy



1.Locarnini S, et al. Antivir Ther. 2004;9:679–93. 2.Locarnini S, et al. Antivir Ther. 2007;12:H15-H23. 3. Ghany M & Liang TJ. Gastroenterology 2007;132:1574-85. 4.Zoulim F, et al. Antiviral Res. 2004;64:1-15 .5. Locarnini S, et al. J Hepatol. 2003;39:S124-S132.

Genetic Barrier for Antiviral Drugs in Nucleoside-Naïve Patients



Genetic barrier increases as the number of specific mutations required for drug resistance increases[6]

1. Locarnini S, et al. J Hepatol 2006;44:422–431. 2. Zeffix® (lamivudine) SmPC. February 2008. 3. Sebivo® (telbivudine) SmPC. June 2007. 4. Hepsera® (adefovir) SmPC. October 2007. 5. Baraclude® (entecavir) SmPC. February 2009. 6. Lok AS, et al. Hepatology 2007;46:254–265. 7. Villet S, et al. J Hepatol 2007;46:531–538.

Ways to Prevent Resistance

Maximize antiviral activity

- increase maximum tolerated dose
- select most effective regimen (combination)
- nucleoside analogue potentiation

Maximize genetic barriers to resistance

- **avoid sequential monotherapy**
- **choose drugs requiring multiple resistance mutations (1 or 2 mutations pre-exist vs 3 or 4 require ongoing selection)**
- **choose drugs where patient is naïve**

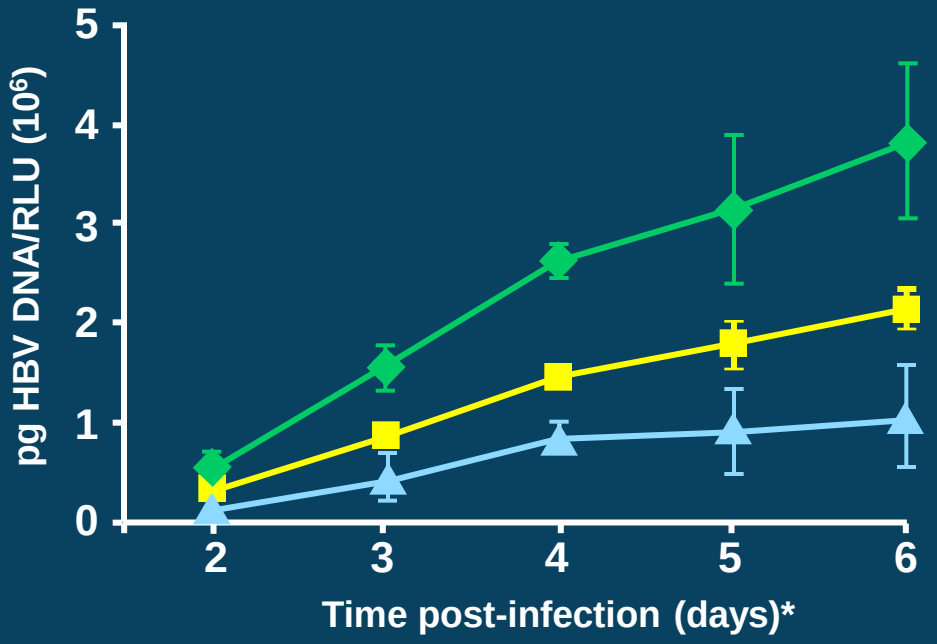
Increase pharmacologic barriers

- patient compliance
- raising trough levels
- Prior drug experience
- Drug metabolism
- pharmacodynamic issues (eg, cirrhosis)

Professor Douglas D. Richman. Hepatology. 2000;32:866

Impact of Substitutions on Viral Fitness

Resistance substitutions usually impair the ability of the virus to replicate[1]



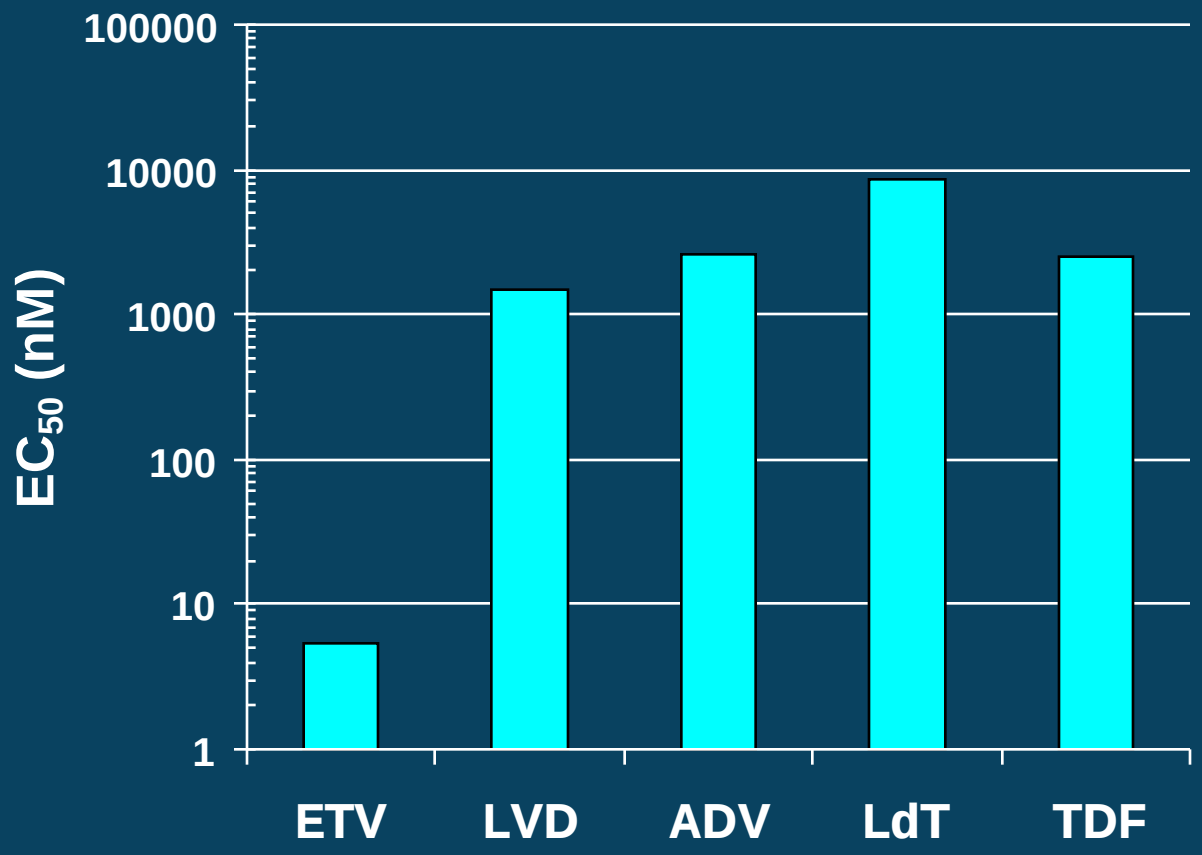
*Transfection of HepG2 cells with recombinant viruses from a patient who was LVD-resistant and experienced virological breakthrough after more than 1 year of ETV therapy[1]

Entecavir susceptibility varies according to the specific resistance substitution and the proportion of variants in the quasispecies[2]

1. Adapted from Tenney DJ, et al. Antimicrob Agents Chemother 2004;48:3498–507. 2. Baldick CJ, et al. J Hepatol 2008;48:895–902.

Comparative EC_{50} for HBV Antivirals in Cell Culture

WT: ETV EC_{50} = 5 nM (> 275-fold more potent)



Langley D, et al. J Virol 2007;81:3992–4001.

Resistance Rates Through 6 Years Among Nucleos(t)ide-Naïve Patients

§ **Patients with HBV DNA ≥400 copies/mL at Week 72 could add FTC to TDF;**

* Cumulative probabilities of resistance taken; † Naïve HBeAg (+); ‡ Naïve HBeAg(-); N/A not available.

1. Locarnini S. Hepatol Int 2008;2:147–151. 2. Lai CL, et al. N Engl J Med 2007;357:2576–2578; 3. Liaw YF, et al. Gastroenterology 2009;136:486–495.
4. Snow-Lampart A, et al. AASLD Oct 30–Nov 3, 2009, Boston, USA. Poster Presentation 480. Hepatology 2009;.532A

Cross-Resistance

- **Resistance to drug(s) to which a virus has never been exposed**
- Resistance-associated mutations selected by drugs may diminish the antiviral activity of other drugs[1]:
  - ***this should be considered before any antiviral drug is prescribed***
- Cross-resistance tends to be more common for compounds sharing structural properties[2]
- Any change in therapy, typically combination or add-on strategies, should be made using drugs that lack cross-resistance with the failing agent[1]

1. EASL Clinical Practice Guidelines Panel. J Hepatol. 2009;50:227-42. 2. Ghany M & Liang TJ. Gastroenterology 2007;132:1574-85

Common Pathways of Antiviral Resistance in CHB: Cross-Resistance

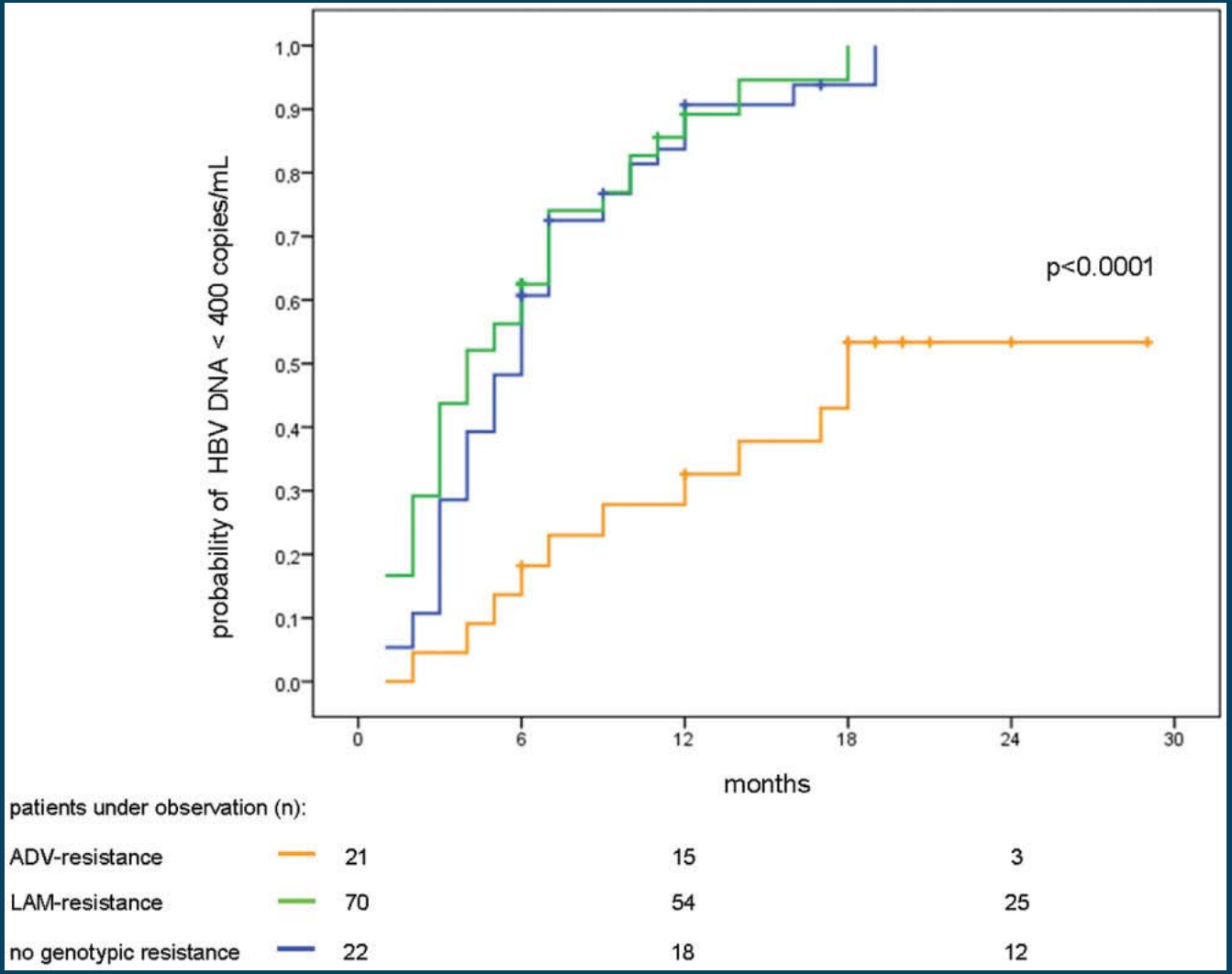
Pathway	Resistance Substitutions	Antiviral Agent	Cross Resistance Profile			
			L-NA	ADV	TDF	ETV
L-Nucleoside (L-NA)	rtM204V/I	Lamivudine (LMV) Emtricitabine (FTC) Telbivudine (LdT)	R	S	S	I
Acyclic Phosphonate	rtN236T	Adefovir (ADV) Tenofovir (TDF)	S	R	I	S
"Shared"	rtA181T	L-Nucleoside Acyclic Phosphonate	R	R	I	S
Naïve Entecavir Resistance	rtL180M+rtM204V with one of rtT184, S202 or M250	Entecavir (ETV)	R	S	S	R
Multi Drug Resistance	Complex patterns e.g. rtA181T+rtI233V+ rtN236T+rtM250L	Multi-Drug	* consult reference laboratory * Role of IFN-α			

R = Resistance; S = Sensitive; I = Intermediate

Modified from Zoulim F and Locarnini S. 2009. Gastroenterol;137:1593

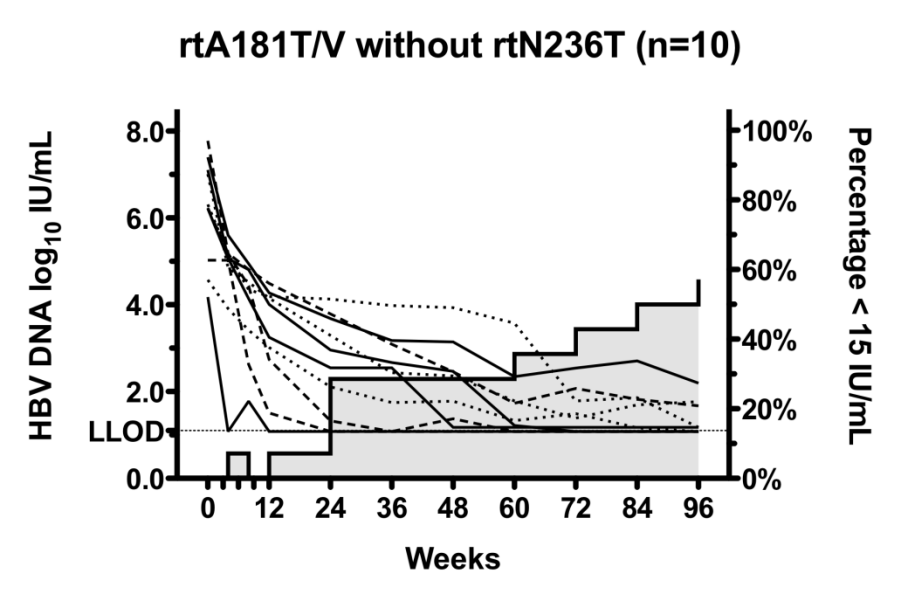
Probability of Achieving Complete Virologic Response Under TDF Monotherapy

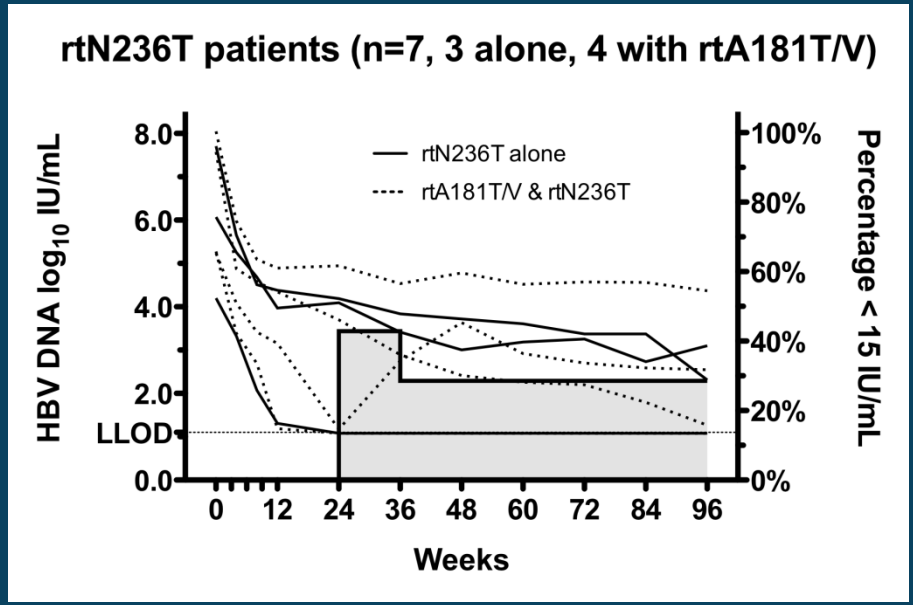
(HBV DNA levels <400 copies/ml)



Van Bommel F. et al 2010. Hepatol;51:73

Tenofovir Rescue Therapy





Patterson, S. et al 2009. Hepatology;50:534A

Practice of Continuing LMV Therapy in Patients with LMV Resistance

Patients with LMV Resistant HBV (rtM204I)

- lower serum ALT compared to pre-therapy
- lower HBV DNA elevations compared to pre-therapy

HOWEVER

- marked flares of serum ALT are observed
- acute exacerbations in liver disease can occur
- these flares may be followed by HBeAg seroconversion and/or immune clearance of mutant HBV

(Liaw et al. 1999. Hepatology 30:567)

BUT

- a new and distinct mutant may be selected and elicit another exacerbation and then select another mutant (**rtA181T/V**)

(Yeh et al. 2000. Hepatology 31:1318)

- Further compensatory mutations continue to occur, such as **rtV173L**

(Delaney et al. 2003. J.Virol 77:11833-11841)

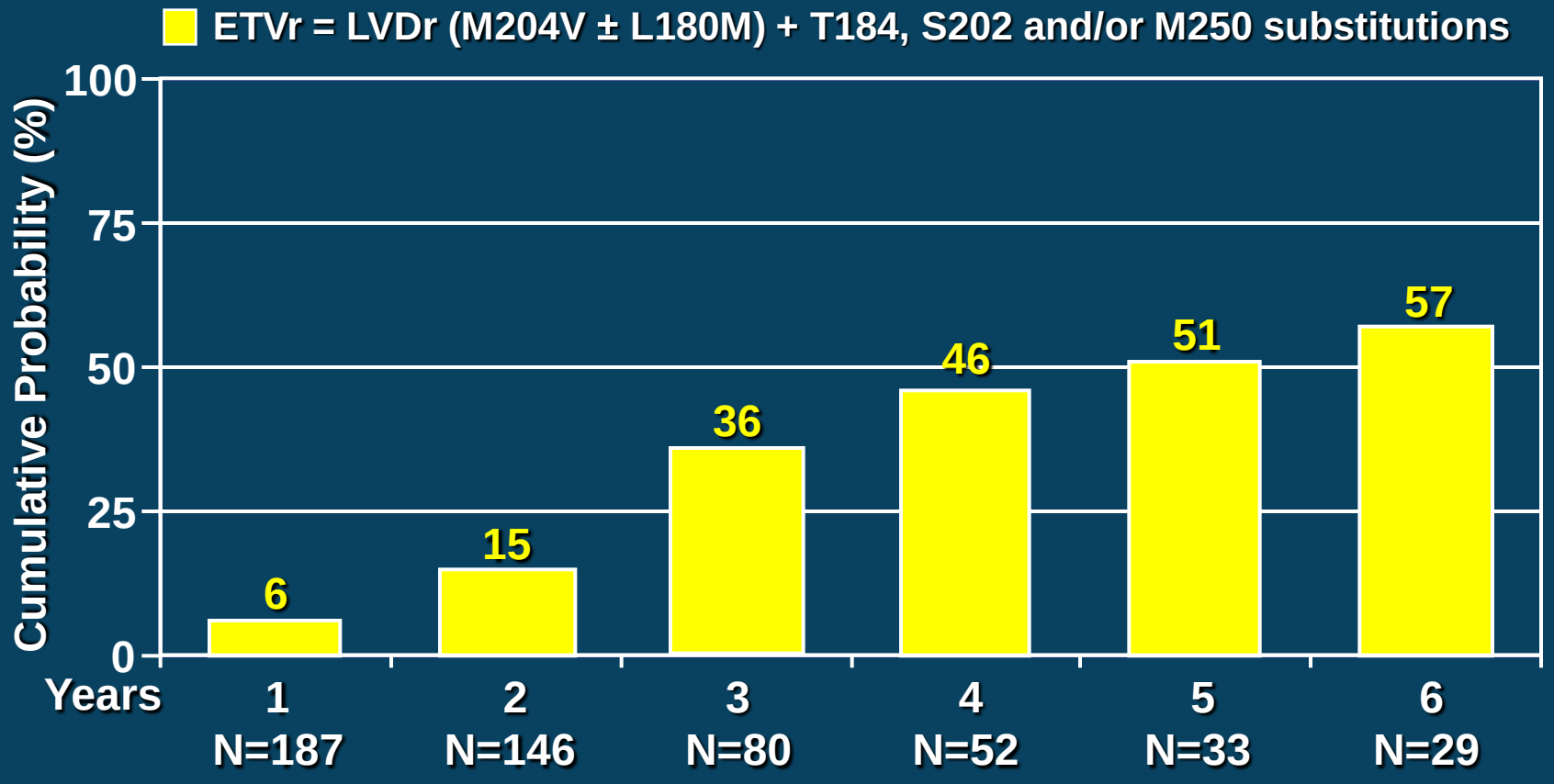
and **rtV214A/rtQ215S**.

(Bartholomeusz et al. 2005. Hepatology 42(Suppl.1):594A)

and **rtT184S**

- Which will affect subsequent efficacy of salvage/rescue therapy

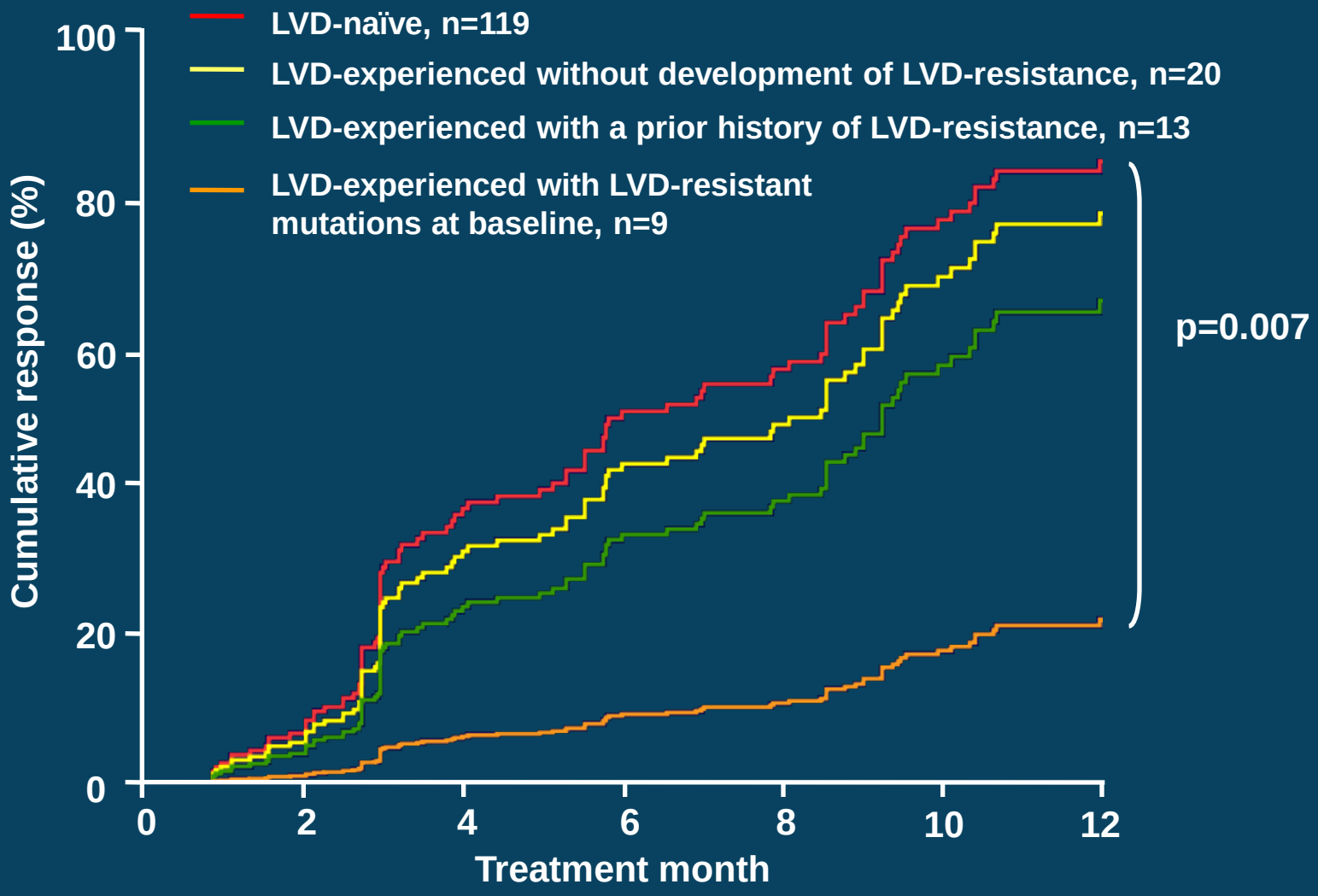
LVD-Refractory Cohort (HBeAg[+]): Cumulative Probability of Entecavir Resistance Through 6 Years



74/187 (40%) achieved HBV DNA <300 copies/mL
5/74 (7%) with HBV DNA < 300 c/mL had subsequent genotypic Entecavir resistance
Majority of patients in this cohort had confirmed baseline LVDr

Tenney, DJ M, et al. 44th EASL, April 22–6, 2009, Copenhagen, Denmark. Oral 20. J. Hepatol 2009;50(S1), S10.

Entecavir: an Option in LVD-Experienced Patients Without LVD-Resistance



Reijnders JGP, et al. 44th EASL, Apr 22-26, 2009, Copenhagen, Denmark. Oral 19. J Hepatol. 2009;50 (suppl 1):S10.

What to do on First Virological Breakthrough/Partial Virological Response

CHECK IF PATIENT COMPLIANT

- Repeat HBV DNA testing in a timely manner to confirm VL breakthrough
- If confirmed [HBV VL ≥ 1.0 log IU/ml] THEN perform HBV POL SEQUENCING
- Typical results for the HBV POL:
  i. "WILD-TYPE" SEQUENCE if no known resistance mutations found
  ii. rtM204V/I±rtL180M detected
  iii. rtA181T/V
  iv. rtN236T±rtA181T/V detected
  v. rtT184S/A/I/L+rtS202G/C (and/or rtM250I/V) WITH rtL180M+rtM204V
  vi. Complex pattern(s) detected

Zoulim, F & Locarnini, S. 2009. Gastroenterology; 137:1593

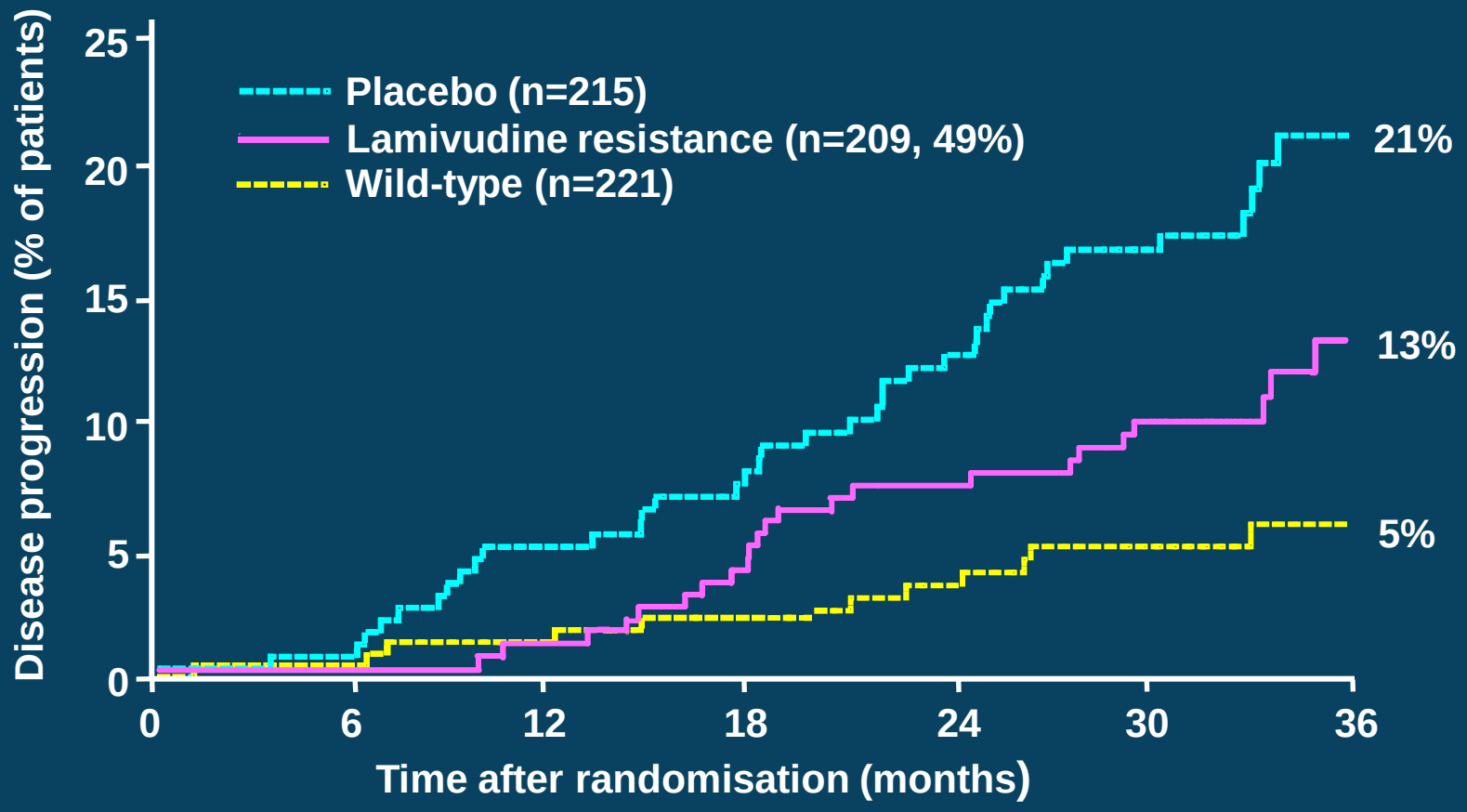
Resistance to Antiviral Therapy can Compromise Treatment Goals

- Development of resistance has a negative impact on CHB therapy:
  - Virological breakthrough[1]
  - ALT elevation or ALT flares[1,2]
  - Decreased HBeAg seroconversion[3]
  - Resistant variants may be archived (cccDNA)[4]
  - Resistant viruses may escape vaccination[5]
  - This may lead to progression of liver disease[6]
    - Hepatic decompensation
    - Hepatocellular carcinoma

Limits Future Treatment Options

1. Lok ASF, et al. Hepatology 2007;45:507-39. 2. Fung S & Lok A. Antivir Ther. 2004;9:1013–26. 3. Leung NWY, Hepatology. 2001;33:1527-1532. 4. Zoulim F, et al. Antiviral Res. 2004;64:1-15. 5. Sheldon J & Soriano V. J Antimicrob Chemotherapy 2008;4:766-8. 6. Pawlotsky JM, et al. Gastroenterology 2008;134:405-15.

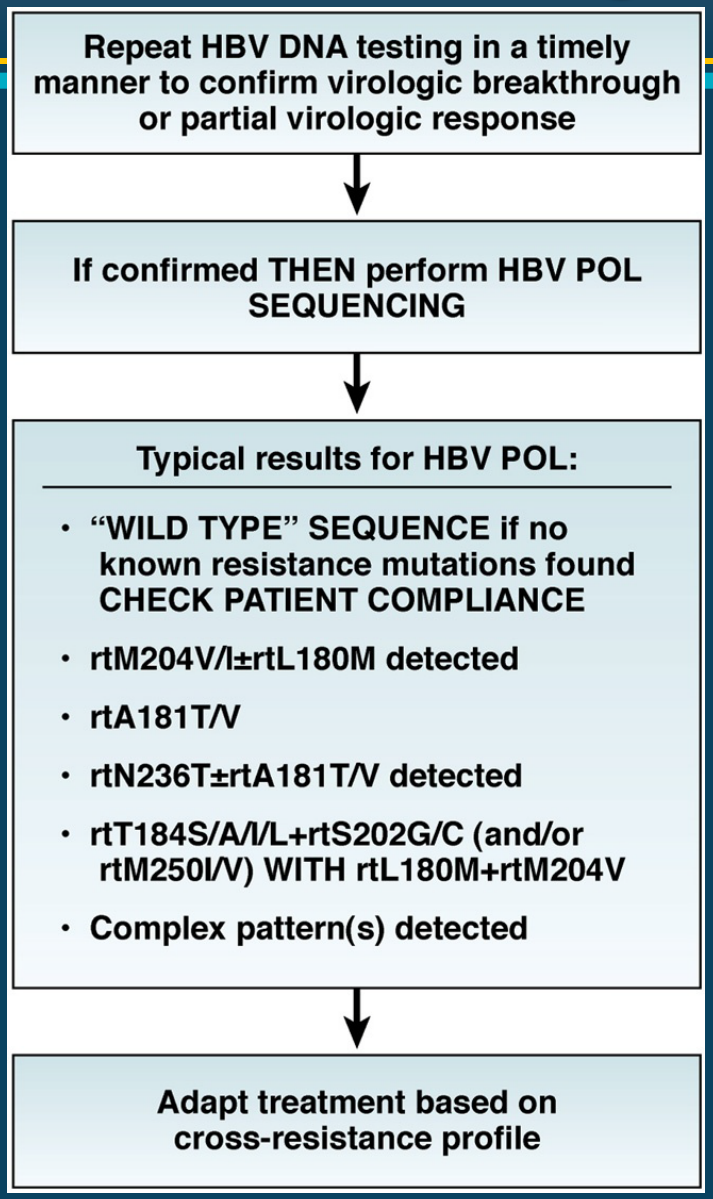
Antiviral Effect on Disease Progression* is Reduced when Resistance Develops



Disease progression, defined by the first occurrence of any of the following: an increase of at least 2 points in the Child–Pugh score, spontaneous bacterial peritonitis with proven sepsis, renal insufficiency, bleeding gastric or esophageal varices, the development of hepatocellular carcinoma, or death related to liver disease.

Adapted from1. Liaw Y-F, et al. N Engl J. Med. 2004;351:1521-31. 2.Liaw Y-F, et al. Semin Liver Dis. 2005;25(Suppl 1):40-7.

Management Flow Chart for First Virologic Breakthrough/Partial Virologic Response



Zoulim F & Locarnini S. 2009. Gastroenterol;137:1593

Cross-Resistance Profile

	Resistance substitution*				
	LVD/LdT-resistant (L180M +/- M204V/I)	ADV-resistant (N236T)	ADV-resistant (A181T/V)	ADV-resistant (N236T+A181T)	ETV-resistant
Mutation confers some degree of reduced sensitivity to listed drugs[1,2†]	▪ Entecavir ▪ Telbivudine	▪ Tenofovir	▪ Lamivudine/ Telbivudine ▪ Tenofovir	▪ Tenofovir ▪ Lamivudine ▪ Telbivudine	▪ Lamivudine ▪ Telbivudine
Drugs remaining fully active[1,3]	▪ Adefovir ▪ Tenofovir	▪ Entecavir ▪ Lamivudine ▪ Telbivudine	▪ Entecavir	▪ Entecavir	▪ Adefovir ▪ Tenofovir

* **First virological breakthrough should be managed with an add-on strategy (combination), not switch (sequential monotherapy).**

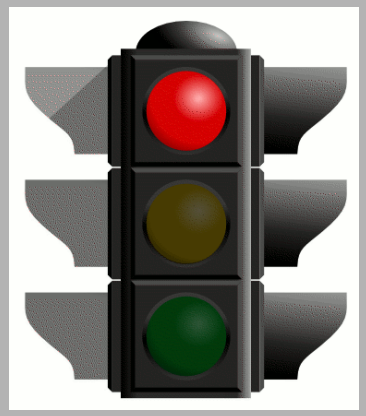
† Impact on sensitivity variable; results according to laboratory analyses, not patient studies.

Resistance profile of tenofovir not determined due to treatment intensification after Week 72.[4,5]

1. EASL Clinical Practice Guidelines Panel. J Hepatol 2009;50:227–242. 2. van Bömmel F, et al. 43rd EASL, April 23–27, 2008, Milan, Italy. J Hepatol 2008;48(Suppl 2):S32. 3. Zoulim F. Antiviral Res 2004;64:1–15. 4. Heathcote J, et al. 59th AASLD, Oct 31–Nov 4 2008, San Francisco, USA. Oral 158. 5. Marcellin P, et al. 59th AASLD, Oct 31–Nov 4 2008, San Francisco, USA. Oral 146.

Zoulim F & Locarnini S. 2009. Gastroenterol;137:1593

Viral Mutational Pathways: rtM204V/I

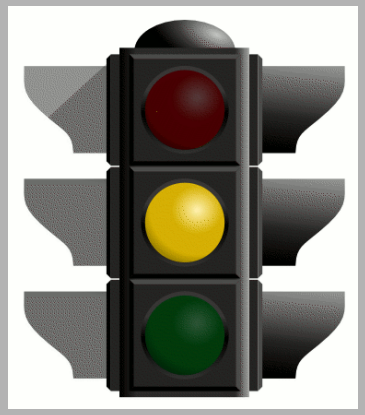


LAMIVUDINE

- not effective

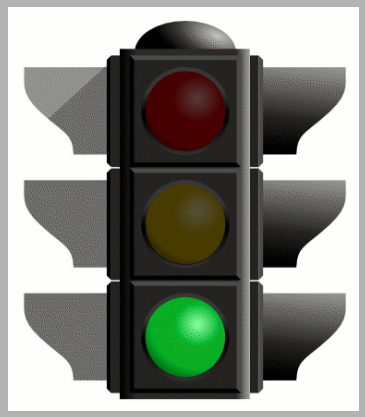
TELBIVUDINE

- not effective



ENTECAVIR

- higher dose needed
- Reduced sensitivity



ADEFOVIR

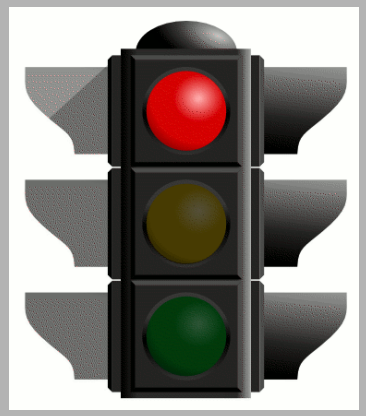
- effective

TENOFOVIR

- effective

rtM204V/I

Viral Mutational Pathways: rtA181T/V



ADEFOVIR

- not effective

LAMIVUDINE

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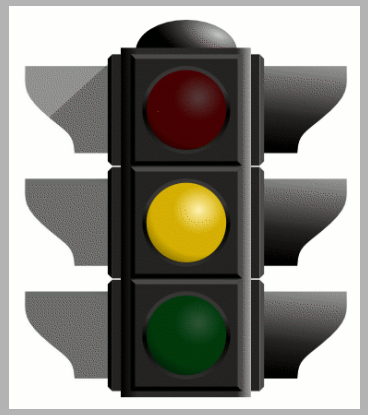
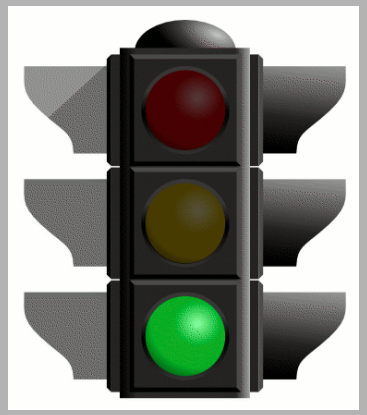
TELBIVUDINE

- not effective



TENOFOVIR

- Reduced sensitivity

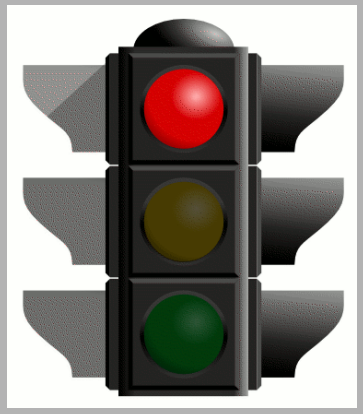


ENTECAVIR

- effective

rtA181T/V

Viral Mutational Pathways:
rtL180M+rtM204V+T184* or S202* or M250*



ENTECAVIR

• not effective

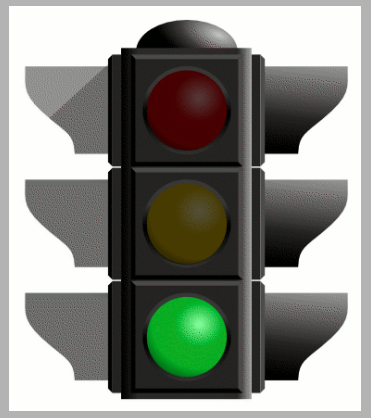
LAMIVUDINE

• not effective

TELBIVUDINE

• not effective

rtL180M+rtM204V
+
T184* or S202* or M250*



ADEFOVIR

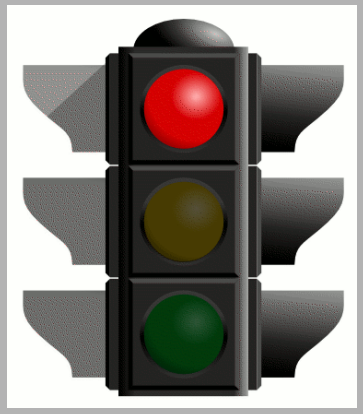
• effective

TENOFOVIR

• effective

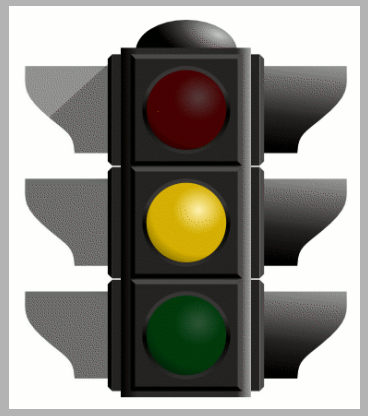
* other aa changes

Viral Mutational Pathways: rtN236T



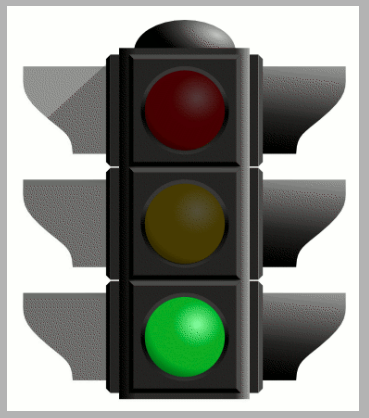
ADEFOVIR

- not effective



TENOFOVIR

- Reduced sensitivity



LAMIVUDINE

- effective

TELBIVUDINE

- effective

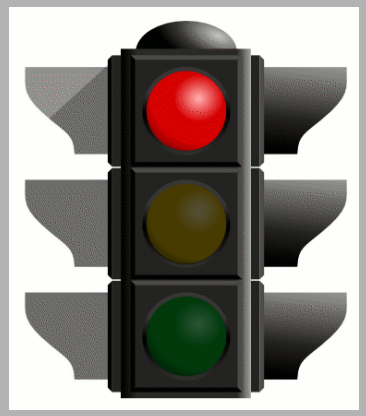
ENTECAVIR

- effective

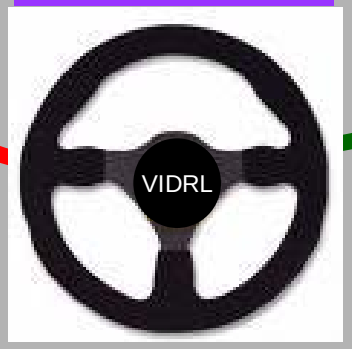
rtN236T

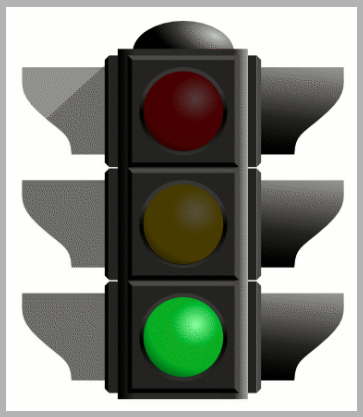
Viral Mutational Pathways:

rtN236T+rtA181T/V



rtN236T + rtA181T/V





ADEFOVIR

- not effective

TENOFOVIR

- not effective

LAMIVUDINE

- not effective

TELBIVUDINE

- not effective

ENTECAVIR

- effective

Transmission of LMV-Resistant HBV

Features

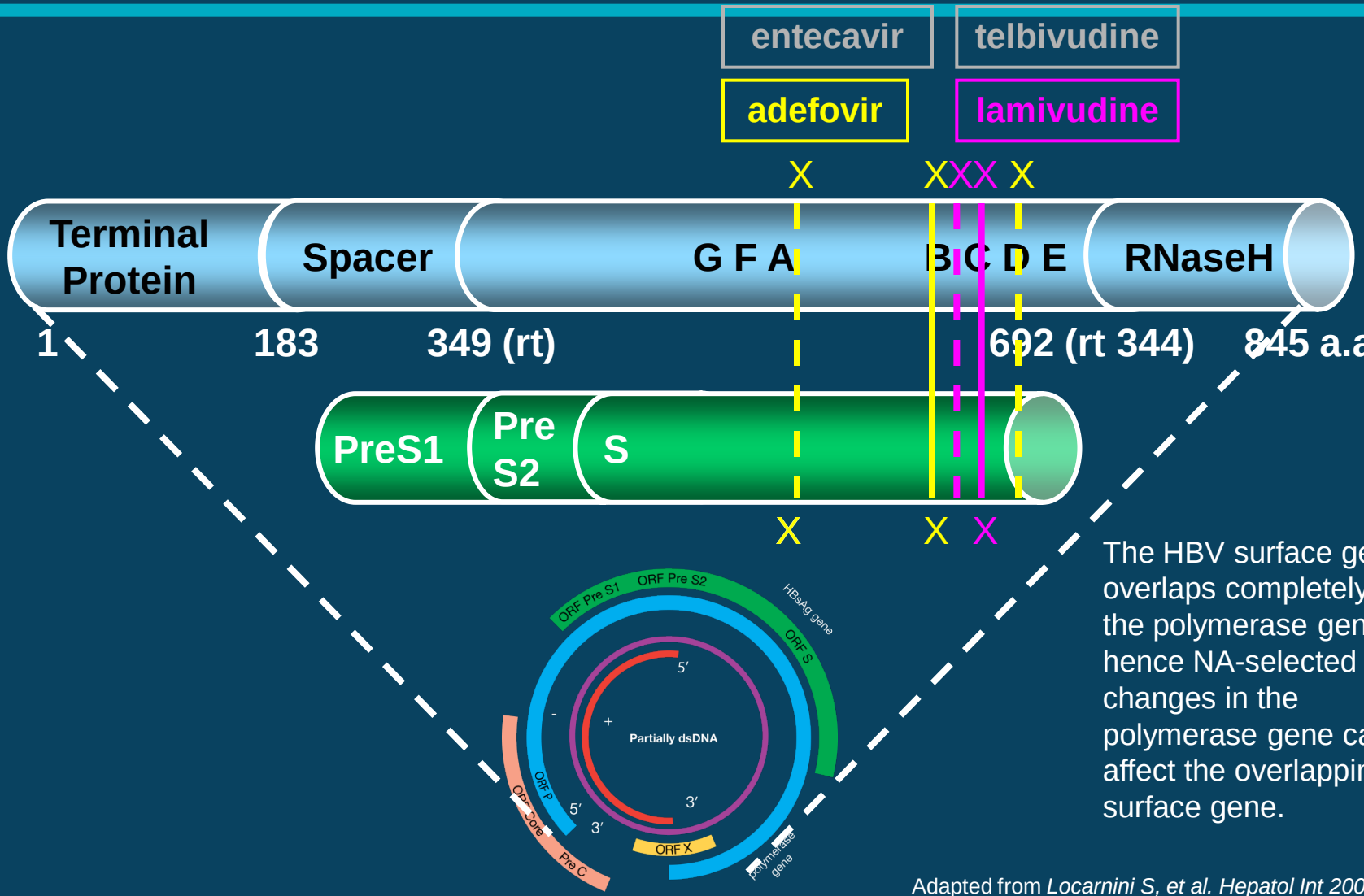
- rtL180M plus rtM204V
- lower viral replication level during acute phase
- incubation period of 2-3 months
- typical acute hepatitis
- not previously vaccinated against HBV

Thibault et al. 2002 AIDS 16:131

- acute hepatitis B caused by LMV-resistant HBV
  - 2/45 cases in Japan

Hayashi, K. et al. 2009. J Gastro Hepatol. In press

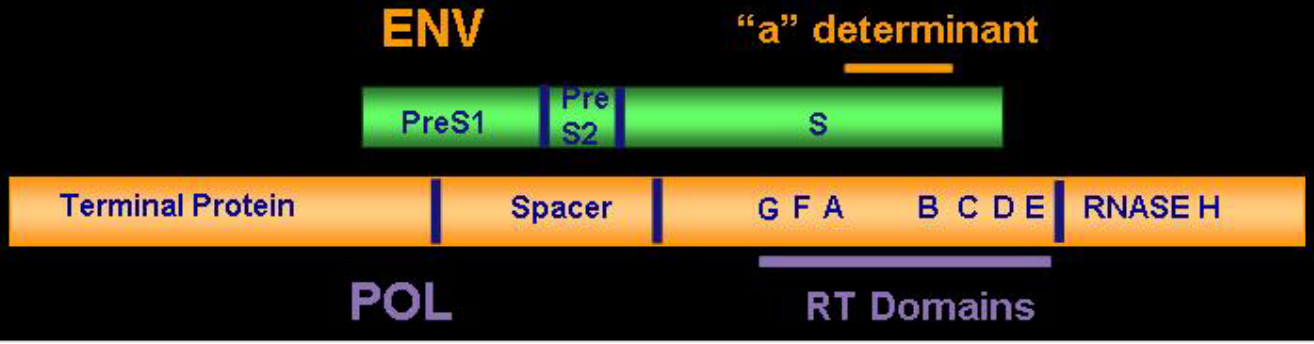
HBV DNA Polymerase-Hepatitis B Surface Antigen Link



The HBV surface gene overlaps completely with the polymerase gene; hence NA-selected changes in the polymerase gene can affect the overlapping surface gene.

Adapted from *Locarnini S, et al. Hepatol Int 2008:2;147–154.*

POL and ENV Link #1



Envelope Mutants	Polymerase Mutant*	Ag-Ab Binding [IC50 (µg/ml)]
Wild type	Wild type	1.09
HBIG-Escape		
sG145R	rtW153Q (W499Q)	>55.0
sD144E/G145R	rtG153E (G499E)	>55.0
Drug Resistant		
sF158Y	rtF166L	1.86
sE164D	rtV173L	14.86
sW196S	rtM204I	8.29
sI195M	rtM204V	5.26
sM198I	rtV207I	12.5
sE164D/I195M	rtV173/rtL180M/rtM204V	54.53

*rtL180M (L526M) in Polymerase causes NO change in envelope.

Torresi, et al. Virology. 2002;293:305.

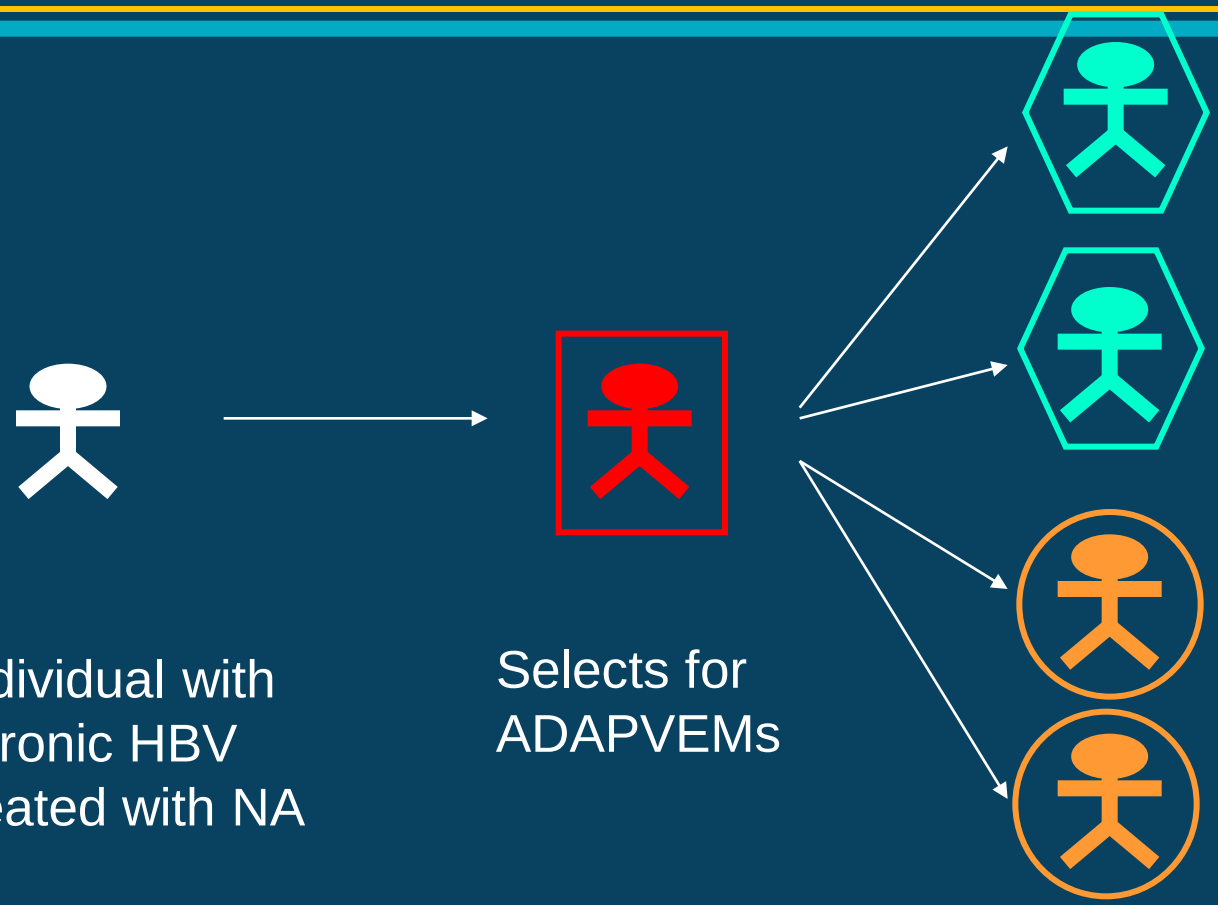
POL and ENV Link #1

Efficacy of Hepatitis B Vaccine Against Antiviral Drug-Resistant Hepatitis B Virus Mutants in the Chimpanzee Model

Saleem Kamili,[1] Vitini Sozzi,[2] Geoff Thompson,[2] Katie Campbell,[3] Christopher M. Walker,[3] Stephen Locarnini,[2] and Krzysztof Krawczynski[1]

2009. Hepatology 49:1483-1491.

Public Health Significance of ADAPVEMs



ADAPVEM: antiviral drug associated potential vaccine escape mutant

Drug-resistant viruses may evade vaccine protection

Prevention of drug resistance is critical

Clements, J. et al 2009. Bull WHO;88:1-80

Strategies to Prevent the Development of Antiviral Resistance

Prevention

- Judicious timing of treatment
- Education regarding adherence to therapy

↓

First line therapy

- High potency drug with high genetic barrier to resistance E.g. Entecavir, Tenofovir
- Consider PEG IFN as an alternative first line therapy (eg. High ALT, Low HBV DNA)

↓

Monitoring

- Regular 3-6 monthly monitoring of viral load with sensitive HBV DNA assay
- Genotypic resistance testing in patients with virological breakthrough

↓

Salvage therapy

- Early initiation of “add on” salvage therapy
- Avoid “switch” sequential monotherapy
- Avoid combination therapy using drugs with similar cross resistance profiles

Summary

- Current emerging patterns of antiviral drug resistance to HBV Pol are complex; But four major pathways can be defined
  - (rtM204V/I;rtN236T;rtA181T/V;ETV [naïve])
- Primary resistance mutations across NA groups: A181T/V
- Broad clusters of compensatory mutations during Lamivudine therapy (T184G/S202I/M250V Vs rtI169T+rtV173L Vs rtT184S) compromising future salvage therapy options with the newer agents (Entecavir)
- Requirement for HBV Pol sequencing to determine profile of antiviral drug resistance
- Emergence of MultiDrug Resistance (MDR) clear cause for concern
- Public health issues around Pol-Env Overlap and vaccine escape
- Need for newer antiviral agents targeted to other sites in the viral life-cycle

Conclusion

Choice of the first line agent is very important in the treatment of CHB!

Questions ?