Mutant problems in Turkey

Ulus Salih Akarca, Ege University, Izmir, Turkey

- HBV and HCV have a very high rate of mutation.
  - HBV reverse transcriptase and HCV RNA polymerase have no proof-reading capacity to correct the misincorporated nucleotides.
  - 10^{10} and 10^{11} mutations may enter the virus pool in HBV and HCV infection, respectively.

- Mutations may accumulate through the evolution and may diversify the subtypes and genotypes. New species may evolve by the accumulation of mutations.
- HBV genotypes have 8%, HCV genotypes have 30% dissimilarities of nucleotide sequence.

- Genotypes have different patterns of clinical progression and treatment response.
- However, we will focus on the importance and consequences of mutations.

- HBV mutations are clinically more relevant comparing to HCV mutations.
  - Vaccination → Vaccine escape mutations
  - Resistance-prone drugs in the treatment
  - Compact genomic structure
- HCV-ISDR (interferon sensitivity determining region) mutations were subjects of several studies, but there has been no consistent results.

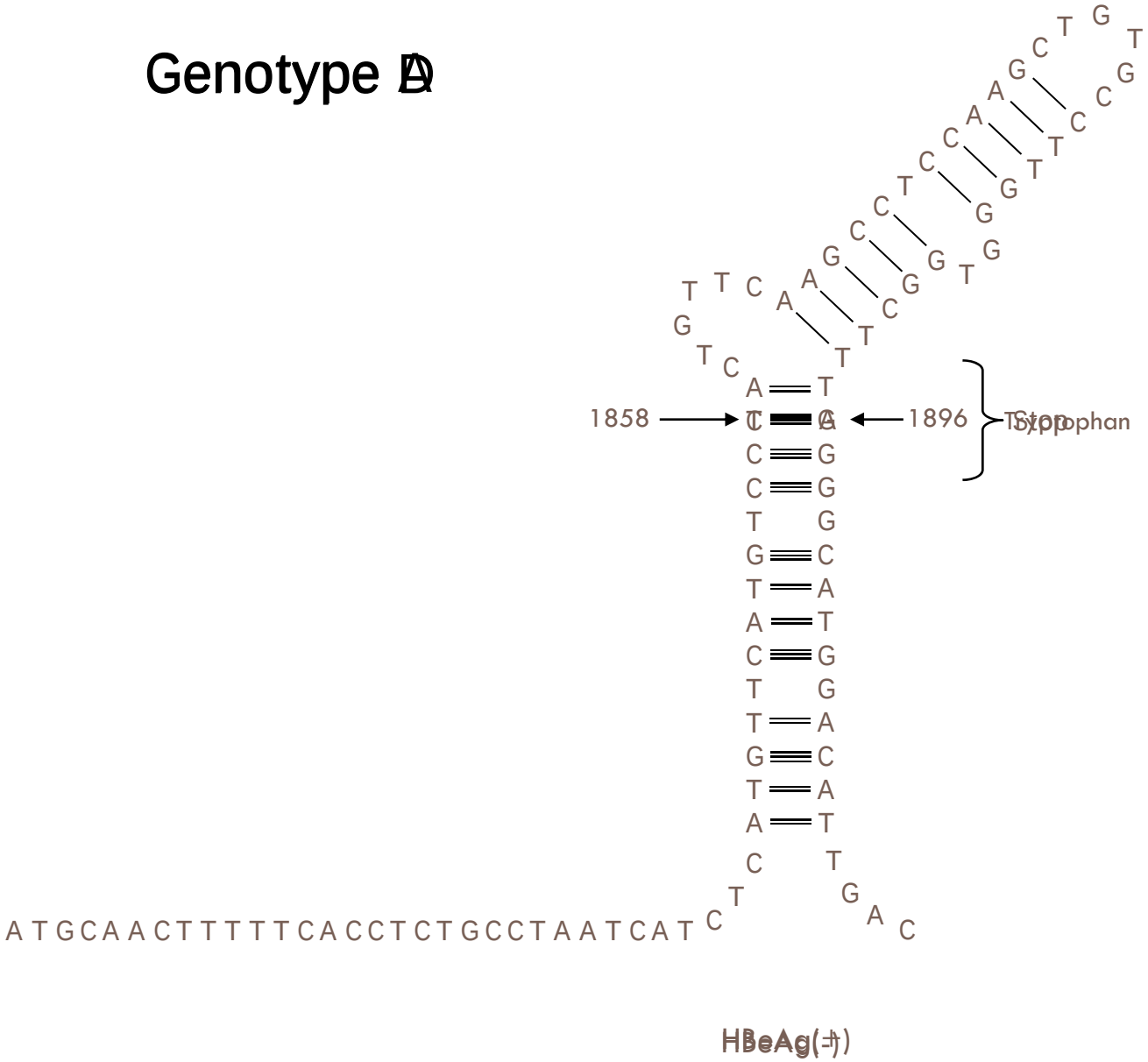
Clinically relevant HBV mutations

- Precore, core promoter mutations which prevent HBeAg secretion.
- Immune-, vaccine-escape mutations of HBsAg,
- Mutations leading nucleos(t)ide resistance.
- Mutations associated with HCC.

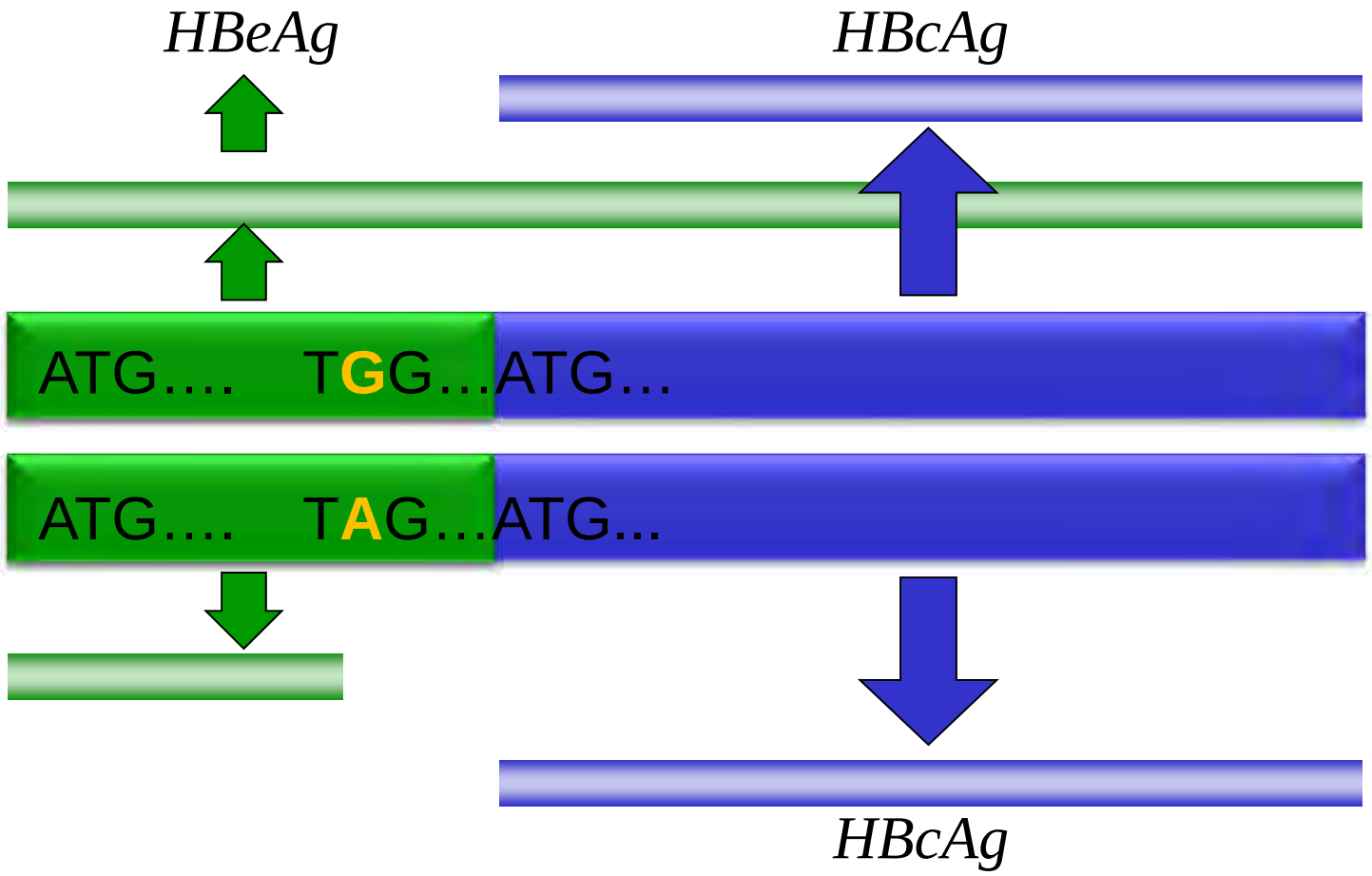
Precore and core promoter mutations

- They should be considered as variations, instead of mutation.
- These mutants are more common than the wild type
- Because of the widespread genotype D infection, precore stop codon mutation is highly prevalent in Turkey

Genotype A


1858
1896
Tryptophan
Stop
A T G C A A C T T T T T C A C C T C T G C C T A A T C A T C
HBeAg(+)
HBeAg(-)

Result of precore stop codon mutation



What are the advantages of HBeAg negative mutants for the virus?

- Escape from immune recognition.
- More effective replication and viral mechanisms
  - Enhance the strenght of ε encapsidation signal sequence
  - Restore the replication competence of nucleoside resistant mutants
  - HBeAg precursor may diminish the encapsidation by competition with HBcAg (dominant negative effect). Absence of HBeAg may increase the replication capacity.
  - Core promoter mutants may increase the expression of core antigen, while reduce the synthesis of HBeAg mRNA.

Characteristics of HBeAg negative infection

- HBeAg negative infection:
  - Represents the advanced stage of the disease.
  - An immune tolerogen (HBeAg) does not exist.
  - Other mutations in HBV genome are more likely to be present.

- For these reasons prognosis and response to treatments of HBeAg negative patients is poor.
  - At the time of diagnosis 30% have cirrhosis.
  - Mortality for 4 years is 20%, Risk of HCC is found to be 14%.

HBeAg negativity in Turkey

- 65-80% of the patients with CHB are HBeAg negative.
- These figures are lower comparing to other Mediterranean country.
- Higher rate of vertical transmission and young age of CHB may be the cause.
  - Median age of treated patients was 37, more than 10 years ago
  - Now it is 43

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Journal of Clinical Virology 21 (2001) 91–101

Journal of Clinical Virology

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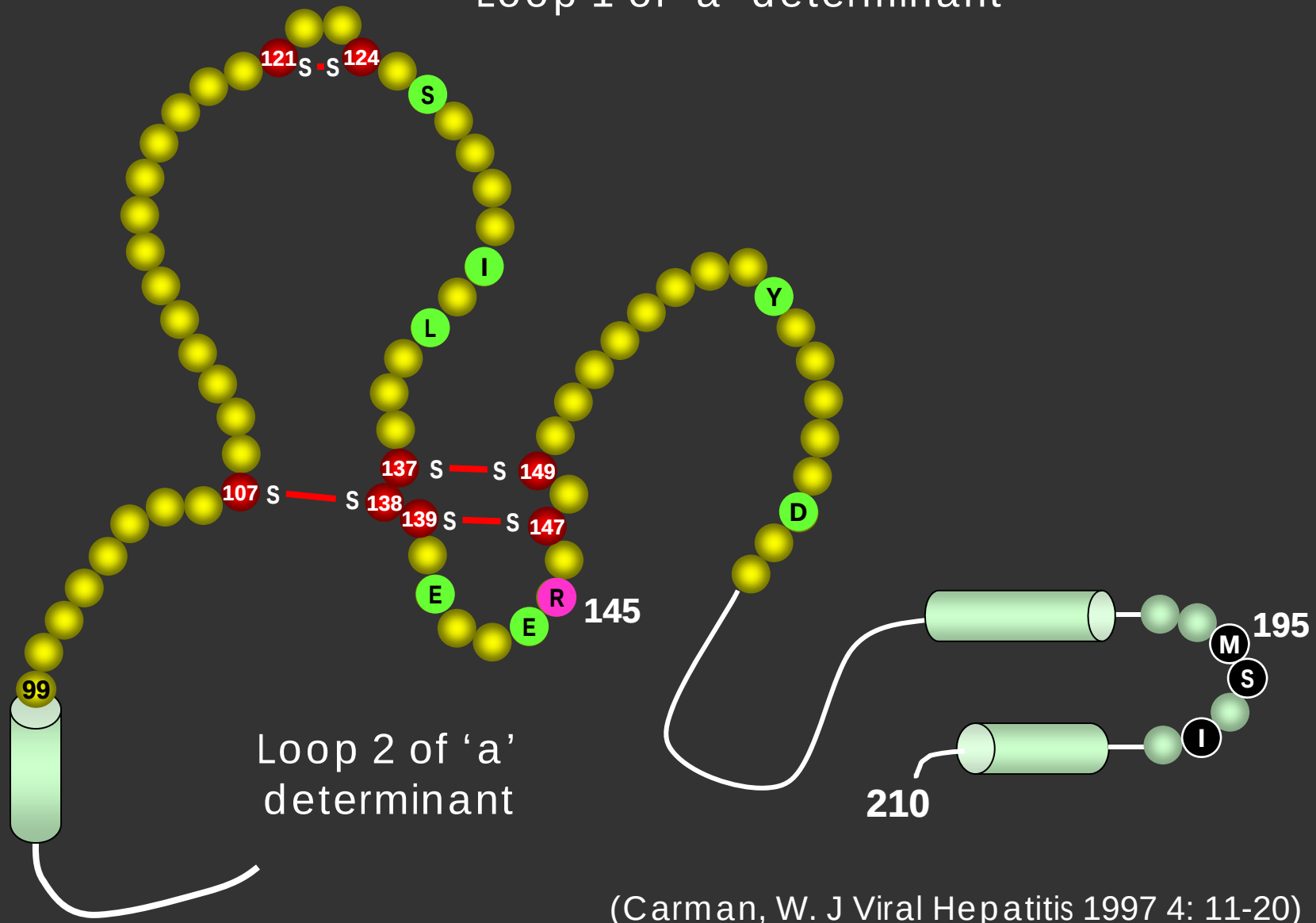
Nucleotide divergences in the core promoter and precore region of genotype D hepatitis B virus in patients with persistently elevated or normal ALT levels

A. Mithat Bozdayı [a,*], Hakan Bozkaya [b], Ahmet Repat Türkyılmaz [a], Mustafa Sarýodlu [b], Hülya Çetinkaya [b], Selim Karayalçın [b], Cihan Yurdaydın [b], Özden Uzunalimoğlu

Table 2
Patient characteristics and core promoter and precore mutations (N:Normal; H: High)

	A1 (HBeAg positive ALT: N)	A2 (HBeAg positive ALT: H)	A1+A2 HBeAg positive	B1 (HBeAg negative ALT: N)	B2 (HBeAg negative ALT: H)	B1+B2 HBeAg negative
No of Patients	13	16	29	20	18	38
Male/Female	8F/5M	2F/14M	10F/19M	10F/10M	4F/14M	14F/24
Age (mean ± SD)	30 ± 10	34 ± 12	32 ± 11	37 ± 11	45 ± 11	41 ± 11
ALT (mean ± SD)	26 ± 10	149 ± 220		22 ± 8	104 ± 66	
Deletion 1763–1770	2	0	2	1	1	2
1762 A→T	1	0	1	0	1	1
1764 G→A	0	1	1	0	2	2
1762/1764 (T/A)	2	3	5	1	2	3
1896 stop codon	2	4	6	8	8	16
1762/1764 (T/A) +1896 (G→A) stop	0	0	0	3	7	10
TOTAL	4	7	11	12	17	29

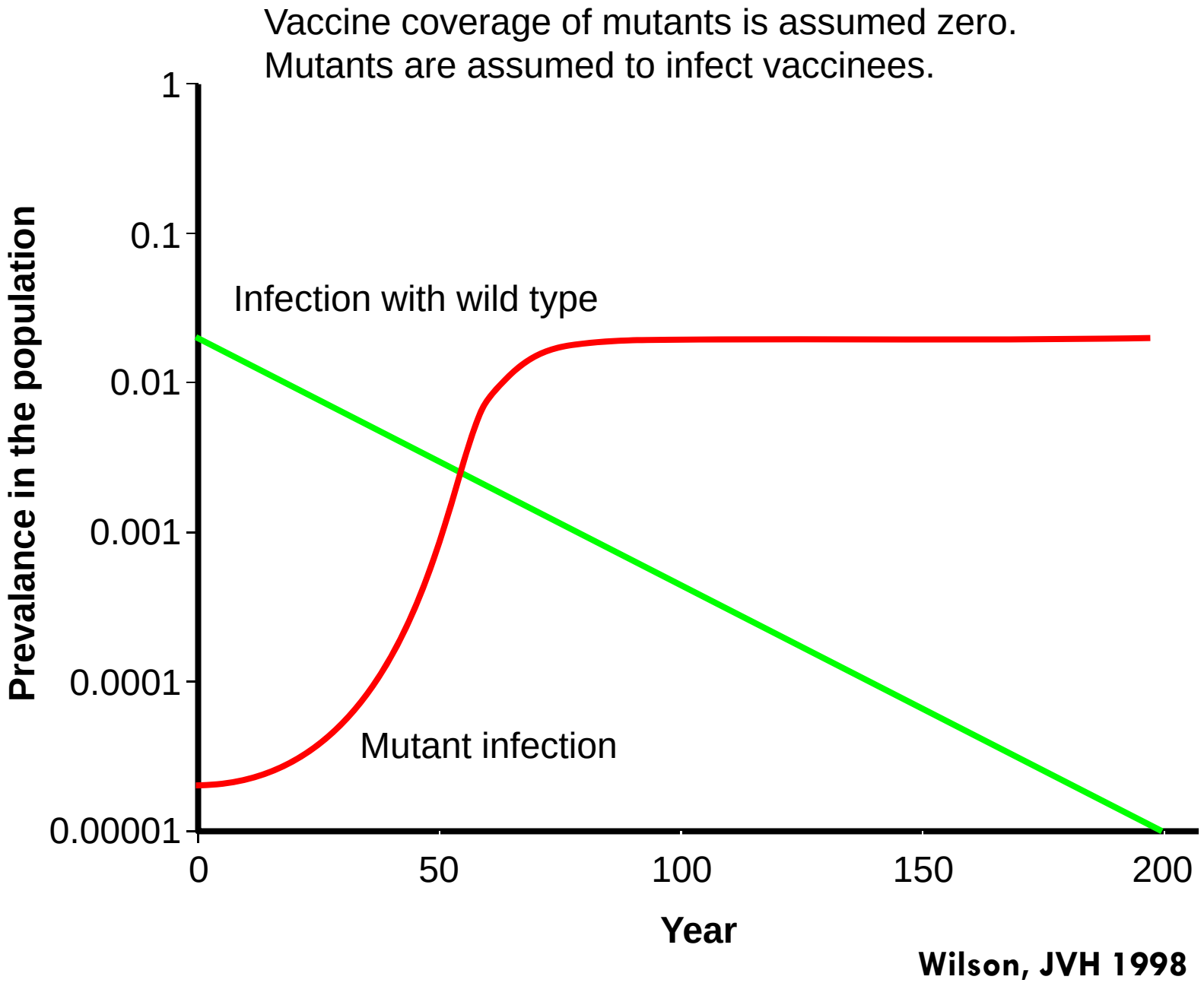
Vaccine esacape mutations in Turkey



(Carman, W. J Viral Hepatitis 1997 4: 11-20)

Clinical consequences of *S*-gene mutations

- HBV infection in postexposure prophylaxis
- HBV recurrence under HBIG prophylaxis in postransplant patients
- Diagnostic inaccuracy in ELISA tests


Vaccine coverage of mutants is assumed zero.
Mutants are assumed to infect vaccinees.
Prevalance in the population
1
0.1
0.01
0.001
0.0001
0.00001
Infection with wild type
Mutant infection
0
50
100
150
200
Year
Wilson, JVH 1998

Publications related to HBsAg mutants from Turkey

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Journal of Clinical Virology 38 (2007) 157–160

JOURNAL OF CLINICAL VIROLOGY

www.elsevier.com/locate/jcv

Short communication

A new hepatitis B virus vaccine escape mutation in a renal transplant recipient

A. Arzu Sayiner[a,*], Harun Agca[b], Aylin Sengonul[a], Ali Celik[c], Mesut Akarsu[d]

A new mutation at a determinant, sS143L, was described. It can not be detected by commercial assay of HBsAg.

Journal of Medical Virology 80:405–410 (2008)

Naturally Occurring MHR Variants in Turkish Patients Infected With Hepatitis B Virus

A. Arzu Sayiner,[1]* Ayla Özcan,[2] and Aylin Sengonul[1]

[1]Department of Clinical Microbiology, Dokuz Eylul University School of Medicine, Izmir, Turkey
[2]Department of Clinical Microbiology, Akdeniz University School of Medicine, Antalya, Turkey

- 41 inactive HBV infection
- 40 patients with CHB were studied.
- 22 patients have aa substitutions.
  - 42.5% in CHB
  - 12.2% in inactive infection
- None of the mutations result in drug resistant changes in rt gene.

TABLE II. Distribution of the Amino Acid (aa) Substitutions of the MHR (aa 100–169) Detected in the Study Population

Sample code	HBsAg subtype	Antigenic regions of MHR													
		HBs1					HBs2	*HBs3*				*HBs4*			
		101	104	110	113	118	120	*125*[a]	*127*	*129*	*136*	*140*	*142*	*143*	*144*
Reference seq. AY796032	ayw2	Gln	Leu	Ile	Ser	Thr	Pro	*Thr*	*Pro*	*Gln*	*Ser*	*Thr*	*Pro*	*Ser*	*Asp*
A11	ayw2						Thr/Pro								
A16	ayw2												*Thr*		
A35	ayw3					Ala									
A38	ayw2	Arg													
A40	ayw2									*His*					
B1	ayw2	Arg													
B3	ayw2						Thr								
B4	ayw3					Ala									
B6	ayw2					Lys									
B9	ayw2	His													
B13	ayw2	Lys									*Ala*				
B14	ayw3							*Met*							
B17	ayw2					Ala			*Ala*						
B18	ayw2													*Leu*	
B20	ayw2			Leu											
B22	ayw2		Trp												
B25	ayw3					Ala									
B31	ayw2			Leu											
B32	ayw3	His			Thr			*Met*							
B35	ayw2														*Glu*
B38	ayw2											*Ile*			
B40	ayw3					Ala									
Number of substitutions at the position (total: 26)		5	1	2	1	6	2	*2*	*1*	*1*	*1*	*1*	*1*	*1*	*1*

Mutations in the *S* gene region of hepatitis B virus genotype *D* in Turkish patients

MEHMET ÖZASLAN[1], ERSAN ÖZASLAN[2*], ARZU BARSGAN[1] and MEHMET KORUK[3]

[1]*Department of Biology, University of Gaziantep, 27310/ Gaziantep, Turkey*
[2]*Department of Gastroenterology, Ankara Numune Education and Training Hospital, 06100 / Ankara, Turkey*
[3]*Department of Gastroenterology, University of Gaziantep, 27310/ Gaziantep, Turkey*

- Isolates from 40 patients were sequenced (nt250-715)
- Mutations were detected at 10 different points.
- C498A, A531G and T536C were observed in all of the isolates.
  - Met125Thr, Thr127Pro aa changes
- C501A point mutation was described in 82.5% of the isolates.
- T496C, C517T, G523A, C479T, T320C (Ser55Phe), C296G (Val47Ala) were found in some patients.
- Most of the family members have the same sequence

Drug resistant mutations in Turkey

HBeAg NEGATIVE PATIENTS WITH CHRONIC HEPATITIS B ARE LESS LIKELY TO HAVE A BIOCHEMICAL BREAKTHROUGH DURING LAMIVUDINE TREATMENT

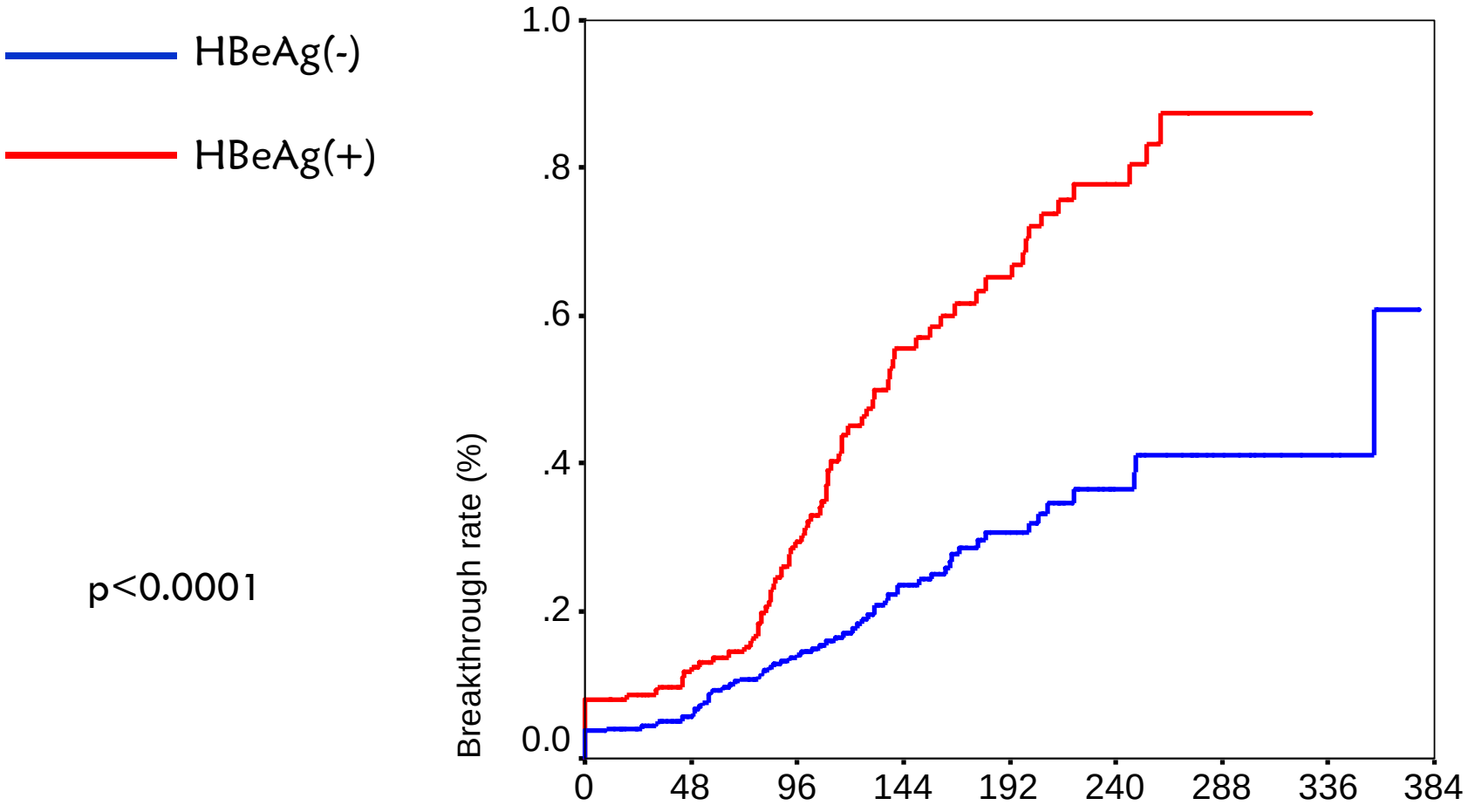
U.S. Akarca[1], F. Gunsar[1], G. Ersoz[1], T. Ozacar[2], S. Erensoy[2], M. Akyildiz[1], E. Kasap[1], S. Akay[1], F. Tekin[1], Z. Karasu[1], Y. Batur[1], T. İlter[1]
[1]: Department of Gastroenterology, 2: Department of Microbiology of Ege University

- 498 lamivudine-treated patients' data were retrospectively analyzed.
- Patients who had two consecutive normal ALT values at least 1 month apart were defined as responsive to treatment.
- Biochemical breakthrough was defined as the presence of two consecutive ALT values more than the upper limit of normal, at least 1 month apart, after ALT normalization.
- Primary resistance was defined as the persistent ALT elevation during lamivudine treatment.
- To investigate the parameters which were related to breakthrough, Kaplan-Meier analysis and log-rank test were performed after categorization of the above parameters. Variables reaching a statistical significance were introduced into Cox's multiple regression analysis.

Characteristics of the patients

Number	498
Gender (male)	344 (69.1%)
Age	40±13
Follow-up (weeks)	105 (0-377)
Response	153 (30.7%)
Basal ALT (IU/l)	
median (range)	113 (34-480)
<2 x ULN	31%
2-5 x ULN	45%
>5 x ULN	24%
Basal HBV DNA (pg/ml)	312 (5-8935)

HBeAg and biochemical breakthrough



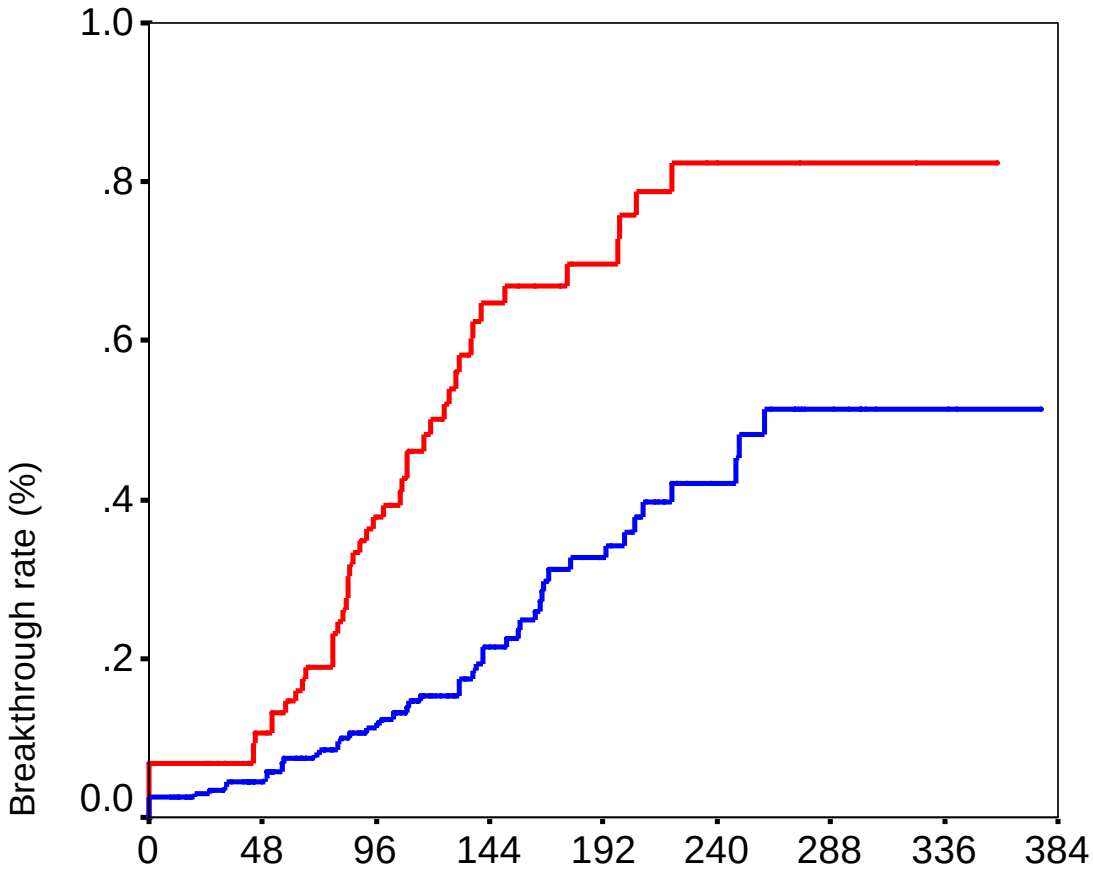
p<0.0001

HBV DNA and biochemical breakthrough

HBV DNA< 2000 pg/ml

HBV DNA≥ 2000 pg/ml

p<0.0001

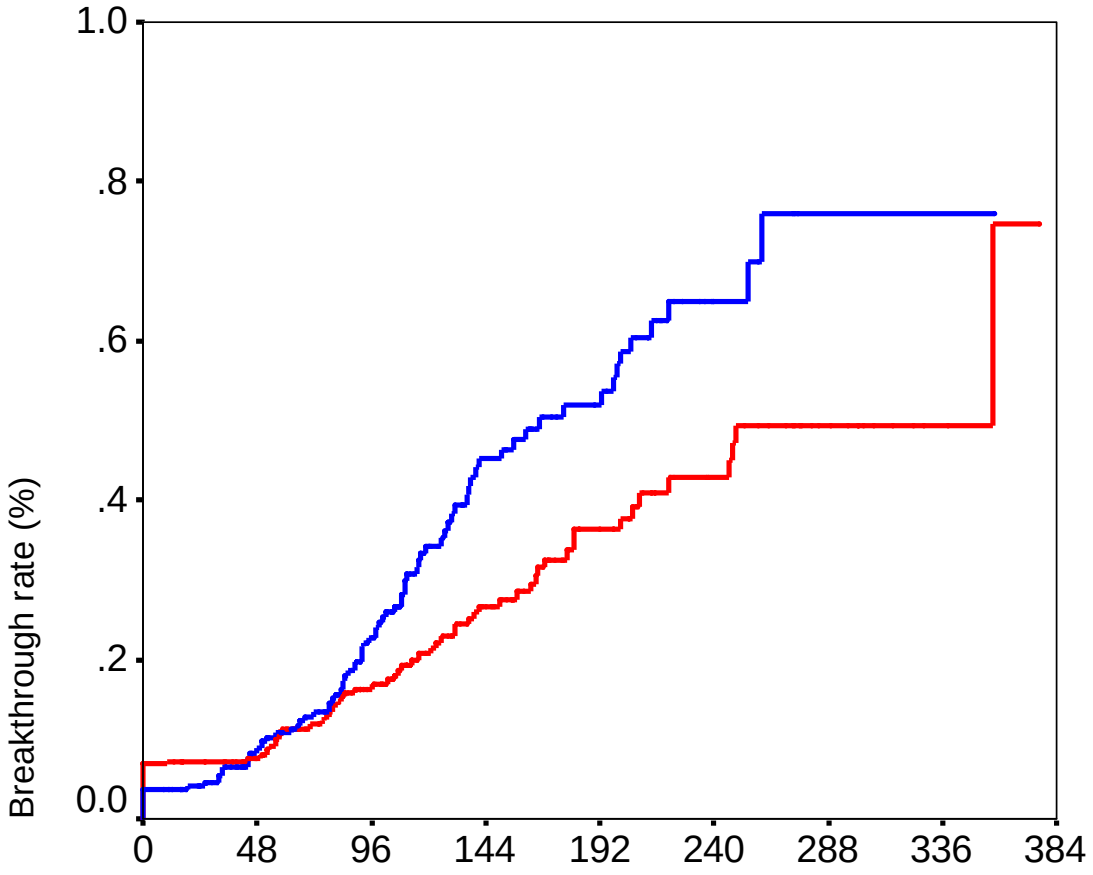


Age and biochemical breakthrough

age<41

age≥41

P=0.0019



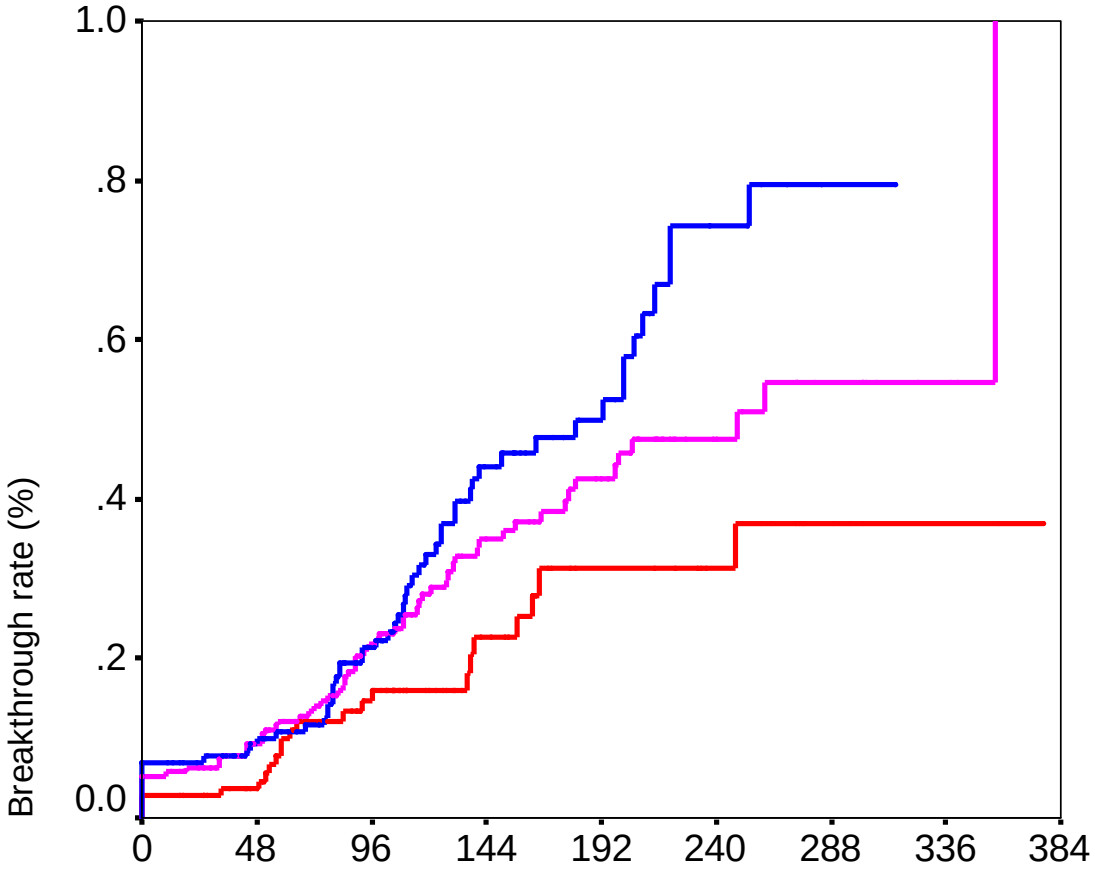
ALT and biochemical breakthrough

ALT<2xULN

ALT 2-5xULN

ALT>5xULN

p<0.0036



- Multivariate analysis indicated that HBV DNA ($p<0.0001$) and categorized ALT (<2xULN, 2-5xULN, >5xULN)($p=0.006$) were independently related to cumulative biochemical breakthrough rate. Decreased rate of lamivudine resistance in HBeAg negative patients did not reach a statistically significant difference ($p=0.158$).

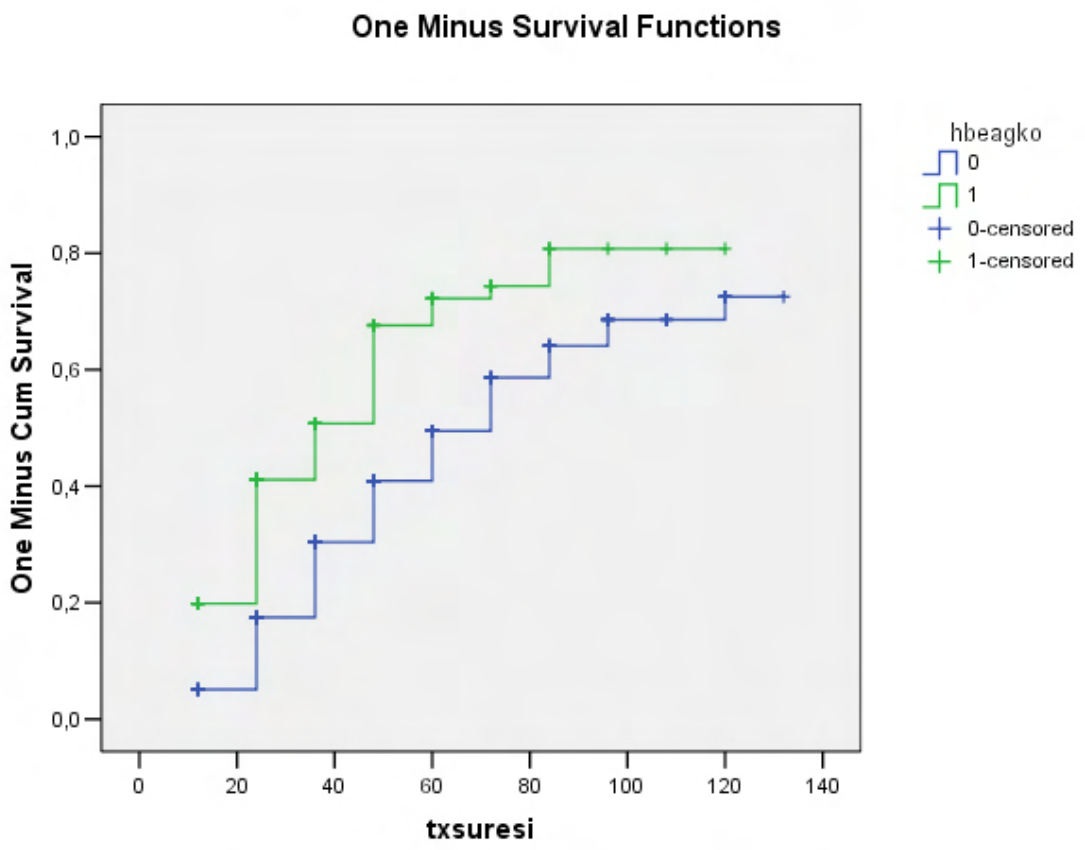
Conclusions

- HBeAg negative patients have a lower rate of biochemical breakthrough during lamivudine therapy comparing to HBeAg positive patients. However, this may be related to lower HBV DNA levels comparing to HBeAg positives.

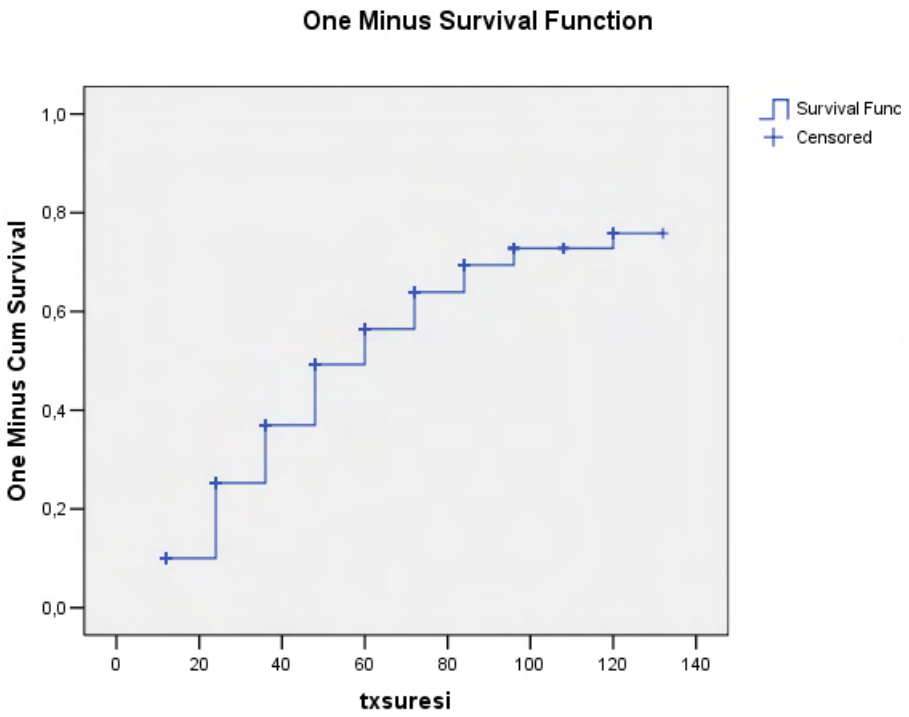
Long-term results of lamivudine treatment for chronic hepatitis B virus infection: 10 year data in HBeAg -positive and -negative patients.

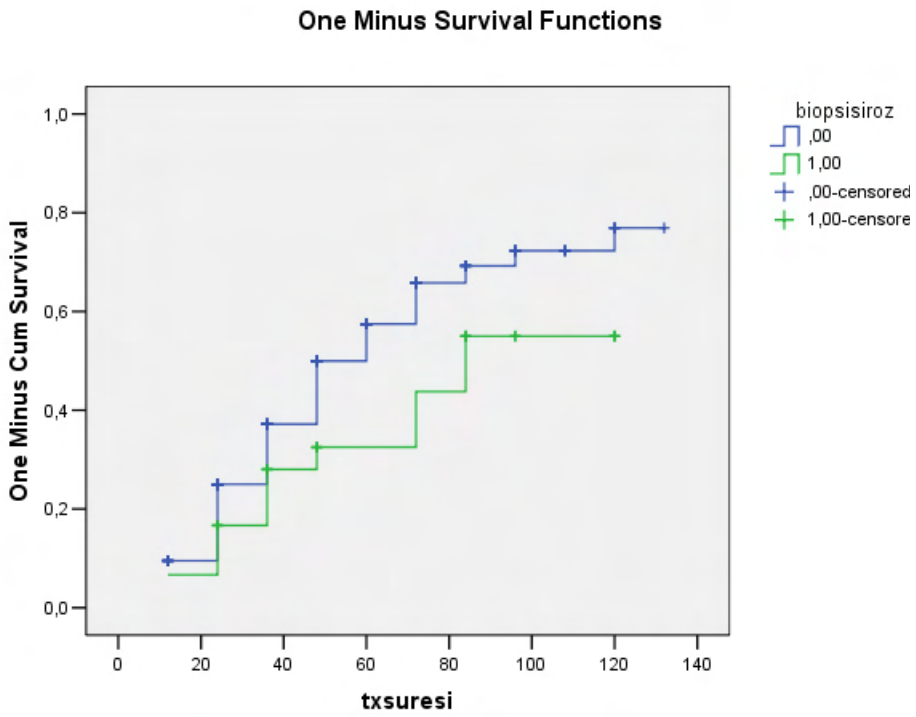
	All patients	HBe Ag (+)	HBe Ag (-)	p value HBe Ag (+) versus HBe Ag (-)
Number of patients	407	131	276	
Age, mean (years ± SD, range)	41.1 ± 12.8 (14–76)	31.9 ± 13.2 (14–76)	43.8 ± 9.3 (18 – 70)	< 0.0001
Male sex (n)	281 (68 %)	87 (65 %)	194 (69 %)	
Number of patients treated with prior interferon therapy	194 (47 %)	71 (53 %)	123 (44 %)	> 0.05
Mean lamivudine therapy duration (months)	41.5 ± 26.1	33.8 ± 22.4	45 ± 27	< 0.0001
Number of patients with cirrhosis	57 (14 %)	11 (8 %)	46 (16 %)	= 0.02
Mean baseline HBV DNA (copy /ml)	$1.7 \times 10^8 \pm 2.4 \times 10^8$	$3.4 \times 10^8 \pm 2.9 \times 10^8$	$7.8 \times 10^7 \pm 1.7 \times 10^8$	< 0.0001
Mean baseline fibrosis score (knodell)	2 ± 1.2	1.6 ± 1.1	2.2 ± 1.2	< 0.0001
Mean baseline ALT levels (IU/L) (range)	87.7± 53.3 (10-425)	88.6 ± 57.5	87.4 ± 51.5	> 0.05

Cumulative proportion of virologic resistances in HBeAg positive and HBeAg negative patients



Cumulative proportion of virologic resistances in cirrhotics and non-cirrhotic patients





Types of lamivudine-resistant mutations

Polymerase mutation	rt204V (n)	rt204I (n)	rt204I+V (n)
rt180L (wild)	6	37	6
rt180M	27	34	8

	All	Serotype		LiPA Genotype		
		HBe-positive	HBe-negative	A	D	Other
L180M	78(68.4%)	37(71.2%)	41(66.1%)	27(71.1%)	24(57.1%)	23(79.3%)
M204I	47(41.2%)	20(38.5%)	27(43.6%)	12(31.6%)	24(57.1%)	9(31.0%)*
M204V	68(59.7%)	33(63.5%)	35(56.5%)	27(71.1%)	17(40.5%)	20(69.0%)**
TOTAL	114(100%)	52	62	38	42	29

Zoulim et al. *J Viral Hepat*. 2006 April ; 13(4): 278–288

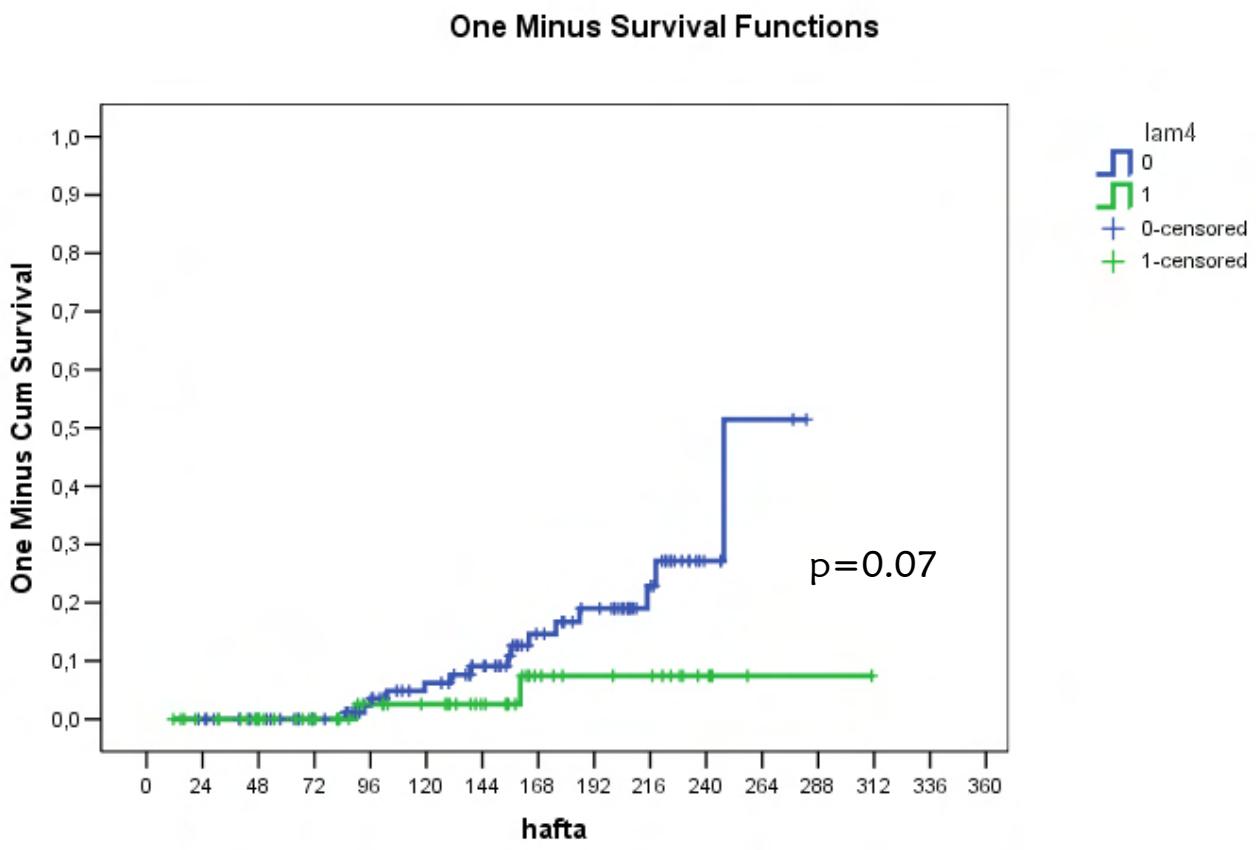
Patterns of lamivudine resistant mutations in lamivudine-treated patients (Bozdayı et al, TASL Meeting 2005)

Mutation patterns	N (315)	%
L180M + M204V	82	26
L180M + M204V/I	11	3
L180M/L + M204M/V/I	12	4
L180M + M204I/V	1	0.3
M204I	79	25
L180M + M204I	45	14
L180M/L + M204I	37	12
M204M/I	34	11
L180M	3	1
L180M/L + M204M/I	8	3
L180M + M204S	3	1
LI80C + M204I	1	0.3
L180M + Y203C + M204I	1	0.3

Adefovir resistance

- 201 lamivudine-resistant patients have been treated with adefovir for more than 1 year.
  - 109 received adefovir monotherapy
  - 92 adefovir add-on to lamivudine
- Median duration of follow-up was 140 weeks
  - 10 pts followed up >240 weeks

Adefovir resistance rate



Adefovir resistance

- 17 patients had adefovir resistant mutations
  - rtN236T 8
  - rtA181V + rtN236T 3
  - rtA181V 3
  - Wt + rtA181T + rtN236T 2
  - Wt + rtA181T/V + rt236T 1

Drug-resistant mutations in treatment-naive patients

HEPATOLOGY

YMDD motif variants in inactive hepatitis B carriers detected by Inno-Lipa HBV DR assay

Mesut Akarsu,* Aylin Sengonul,† Ethem Tankurt,* Ayca Arzu Sayiner,† Omer Topalak,* Hale Akpinar* and Yusuf Hakan Abacioglu†

Departments of *Gastroenterology and †Microbiology and Clinical Microbiology, Faculty of Medicine, Dokuz Eylul University, Izmir, Turkey

- YMDD variants were detected in 13 (18.3%) of the 71 anti-HBe positive inactive HBV carriers. Of the 13 patients, 10 (76.9%) also had accompanying L180M mutation.
- This study used INNO-LIPA. Entecavir or adefovir mutations were not investigated.

Journal of Gastroenterology and Hepatology 2006; **21:1783-1788.**

Summary

- HBV mutation patterns and problems are not different in Turkey from the rest of the world.
- Dominance of genotype D determines the higher prevalence of HBeAg negative infection.
- Precore stop codon mutation is mostly responsible for HBeAg negativity.
- Vaccine escape mutations of surface gene seem not to be an important public health problem.
- Drug resistant mutations have the same rate and same type as the other countries.