版权声明

Wuhan， Sept. 25, 2016

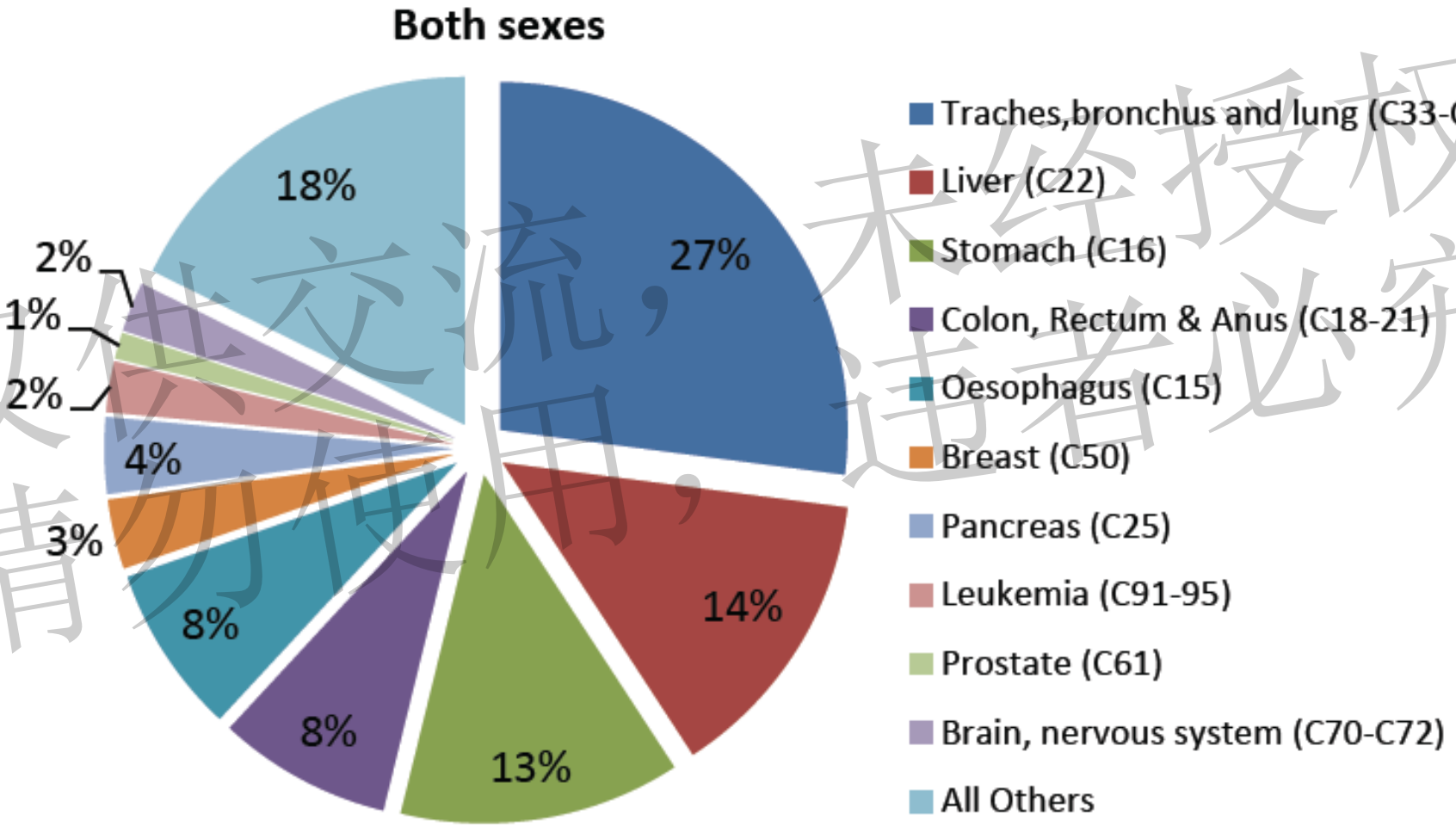
Mechanisms of HBV-induced hepatocarcinogenesis: role of Cancer *Evo-Dev* in the prediction and specific prophylaxis of hepatocellular carcinoma

Guangwen Cao, MD, PhD

Professor, Chairman,

Second Military Medical University

Currently, liver cancer is the 2nd most common cause of cancer death in Mainland China

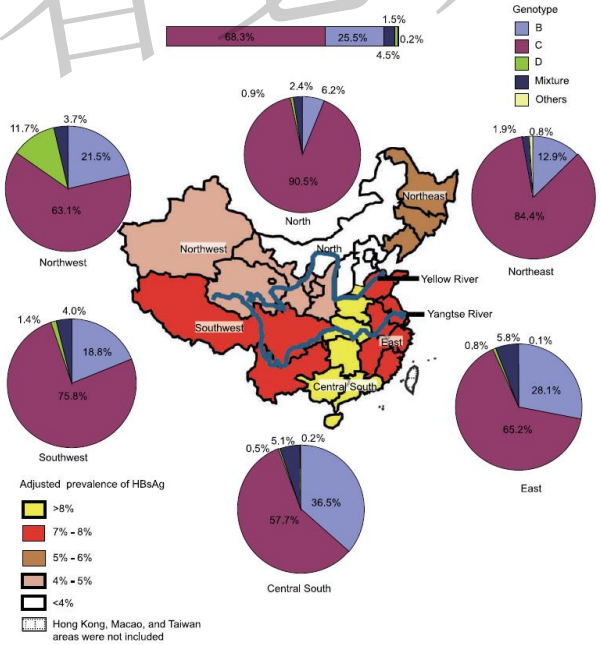


Key epidemiologic data of HCC in Mainland China

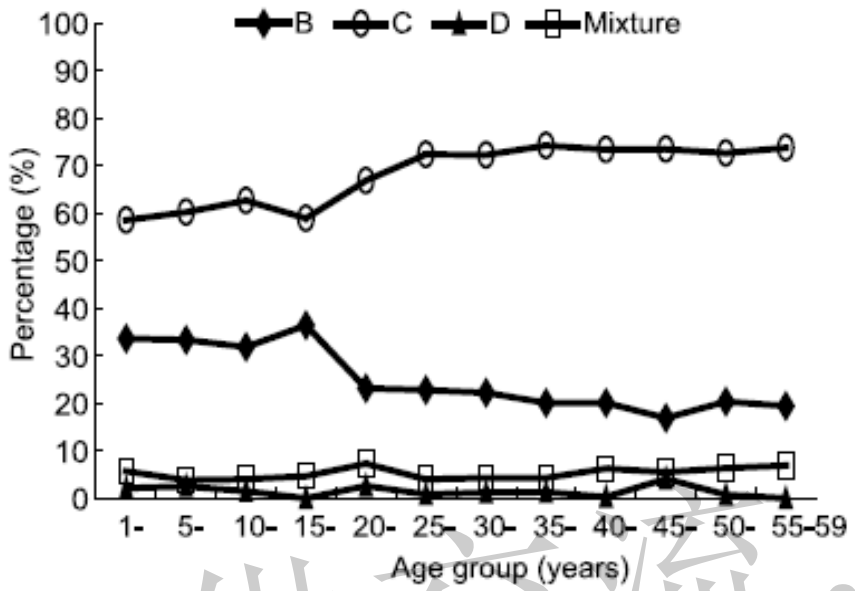
- **HCC accounts for 80% of liver cancer; 90% HCC are contributed by HBV infection**
- **Population accounts for 1/5， HBV carriers for 1/3, HCC for 1/2 worldwide**
- **Among HBV-infected subjects, 32% males and 9% females will develop HCC in 30-75 years old； 20 millions HCC in next 50 yrs**
- **5-year survival is 32% after curative surgery, 9% for non-surgery patients；**

HBsAg seroprevalance in Mainland China in 2006

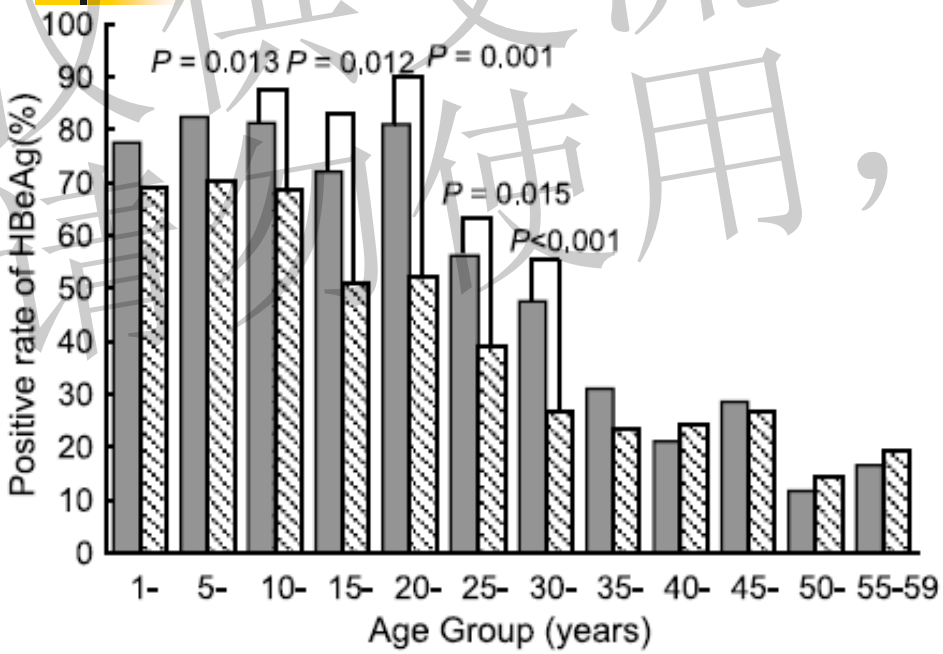
Distribution of HBV genotypes in Mainland China in 2006



Cancer Epidemiol Biomarkers Prev **2010**;19(3):777-86.
J Clin Virol **2007**;38(3):238-43.



Difference in viral replication between HBV genotype B and C, indicating different potential of causing liver disease.



Genotype B2: acute infection, HCC recurrence

Genotype C2: cirrhosis and HCC at late stage

Cancer Epidemiol Biomarkers Prev **2010**;19(3):777-86.

Carcinogenesis **2008**;29(9):1685-91

HBV B2 is apt to cause acute infection；HBV C2 is apt to chronification following an acute course

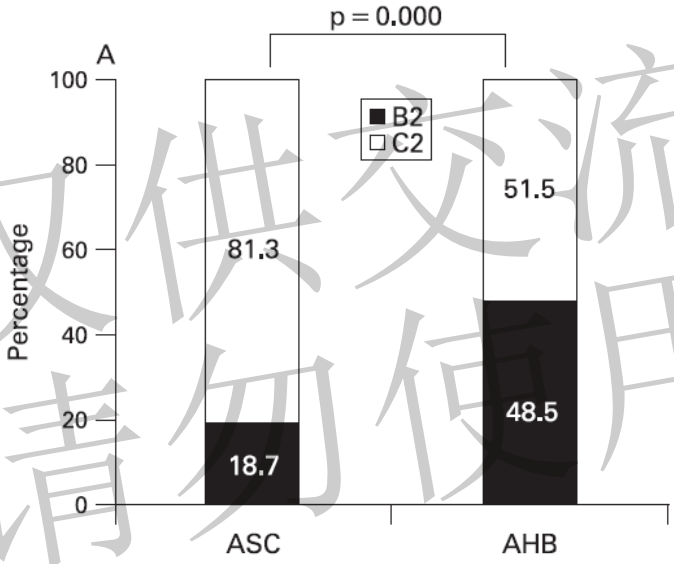


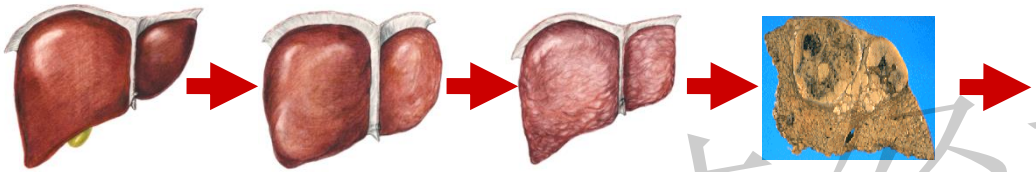
Table 3 Univariate and multivariate regression analyses of the risk factors for chronicification of acute hepatitis B in the patients with hepatitis B virus (HBV) genotyped

	Progression to infection*	Without progression†	OR (95% CI)	AOR (95% CI)
Sex				
Male	19	50		
Female	6	11	1.44 (0.47 to 4.43)	2.61 (0.71 to 9.58)
Age (years)				
⩽40	11	36		
>40	14	25	1.83 (0.72 to 4.69)	0.66 (0.19 to 2.23)
Genotype				
B2	5	31		
C2	20	30	4.13 (1.37 to 12.43)	6.97 (1.59 to 30.63)

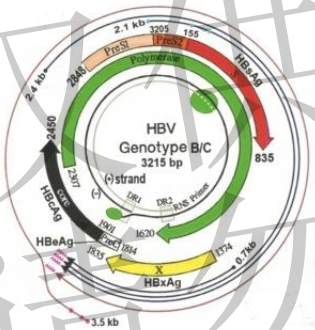
*Progression to chronic HBV infection (n = 25). †Without chronic HBV progression (n = 61)
AOR, adjusted odds ratio; CI, confidence interval; OR, odds ratio.

***Gut* 2008**; 57(12):1713-20. ***Gut* 2009**; 58(7):1028-9.

HBV mutation is a key biomarker during HBV-induced hepatocarcinogenesis

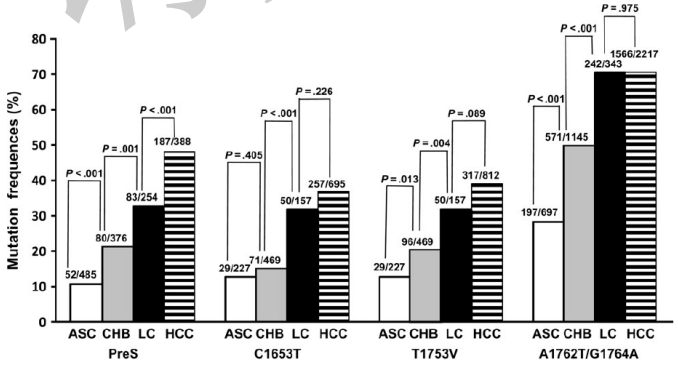


HBeAg (+) ⟶ HBeAg (-)



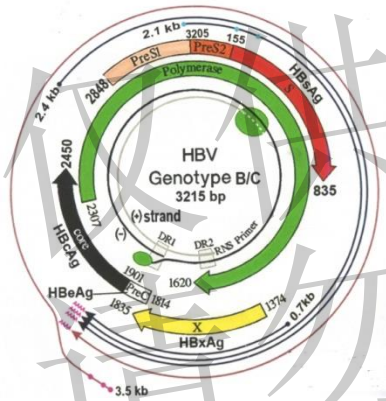
HBV wild-type ⟶ HBV mutation

CD8^{+}T Epitope deletion



J Natl Cancer Inst **2009**;101(15):1066-1082.

HBV mutations in the core promoter region and their associations with HCC and cirrhosis



HBV B2

HBV C2

ASC

CHB

LC

HCC

Mutations at 1674, 1719, 1762, 1764, 1846, 1896, and 1913 in genotype C

Mutations at 1652, 1673, 1674, 1719, 1727, 1730, 1762, 1764, 1766, 1768, 1773, 1799, and 1896 in genotype B

Mutations at 1673, 1726, 1727, 1730, 1764, 1766, 1768, 1773, 1799, and 1896 in genotype C

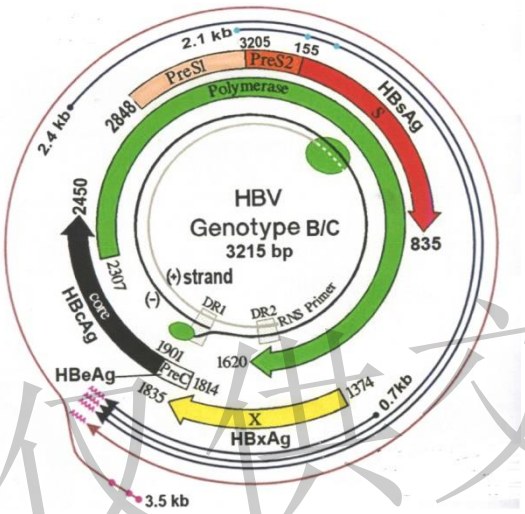
Mutations at 1674, 1762, 1764, and 1896 in genotype B

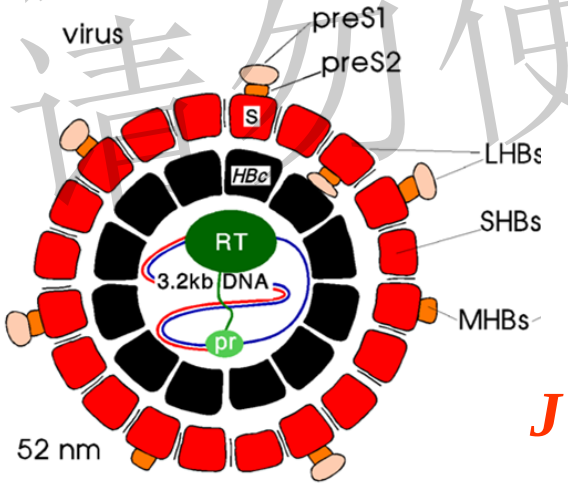
Mutations at 1674, 1753, 1762, 1764, and 1896 in genotype C

Mutations at 1653, 1674, 1719, 1753, 1762, and 1846 in genotype C HBV infected patients seronegative for HBeAg

Am J Gastroenterol **2011;**106(1):81-92.

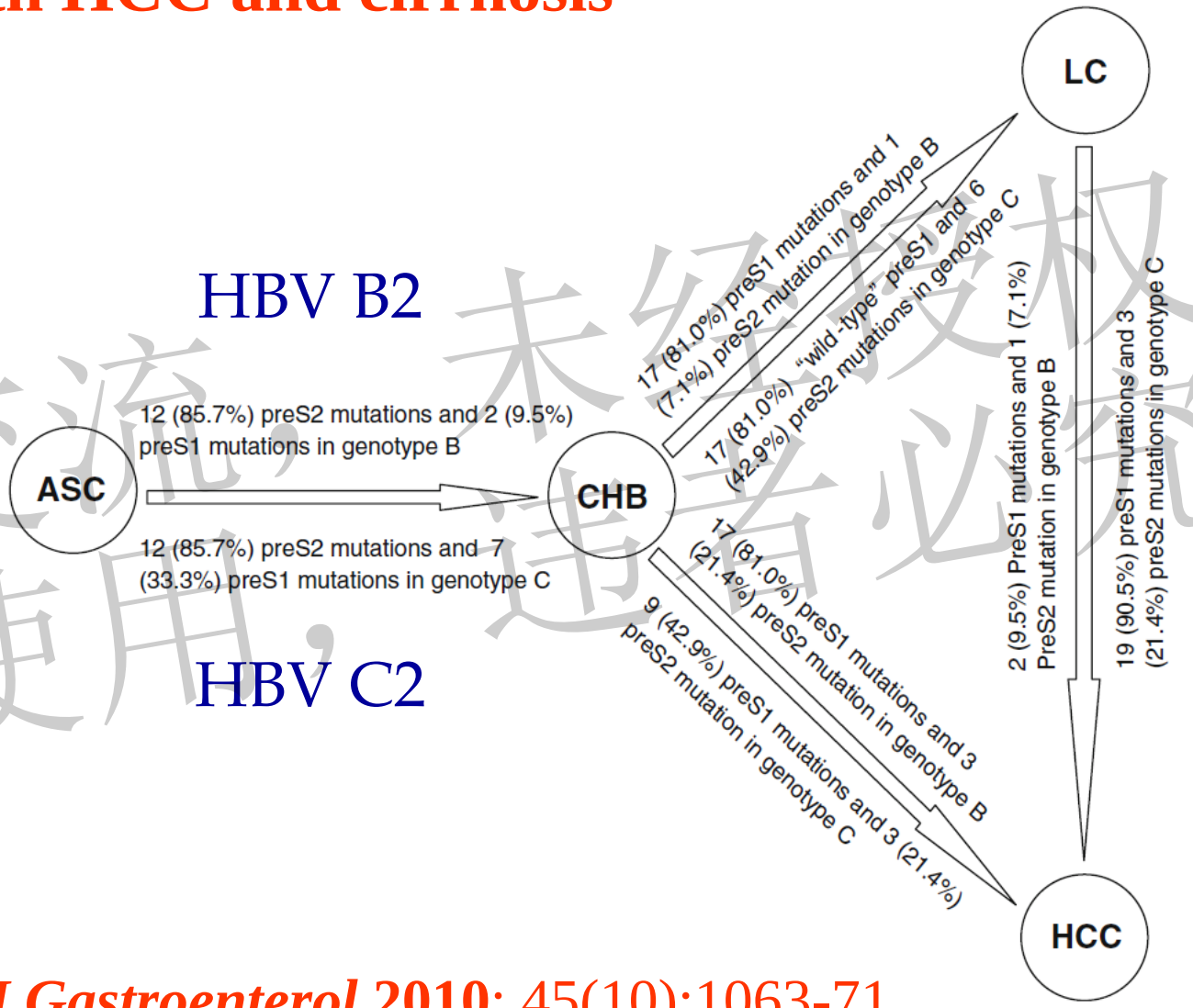
HBV mutations in the preS region and their associations with HCC and cirrhosis





HBV B2

HBV C2



J Gastroenterol **2010**; 45(10):1063-71 .

J Gastroenterol **2011**; 41(1):125-7.

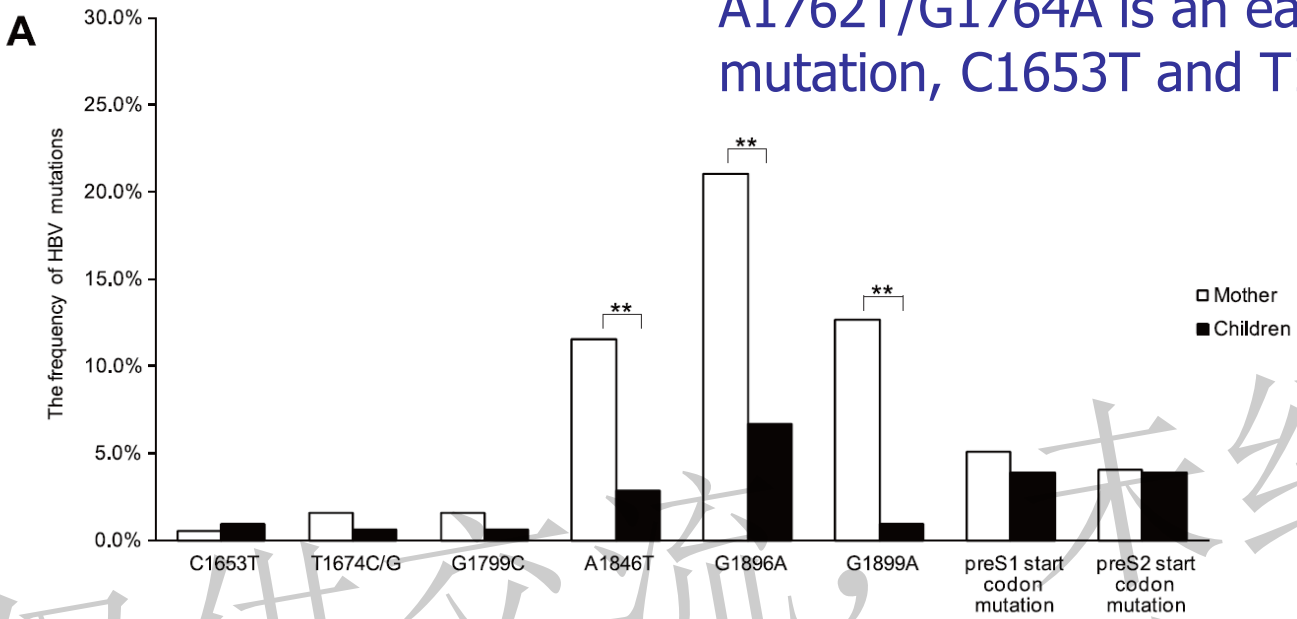
During mother-to-child transmission of HBV, HBV variants can pass placenta barriers; wildtype HBV has advantage of infecting infants

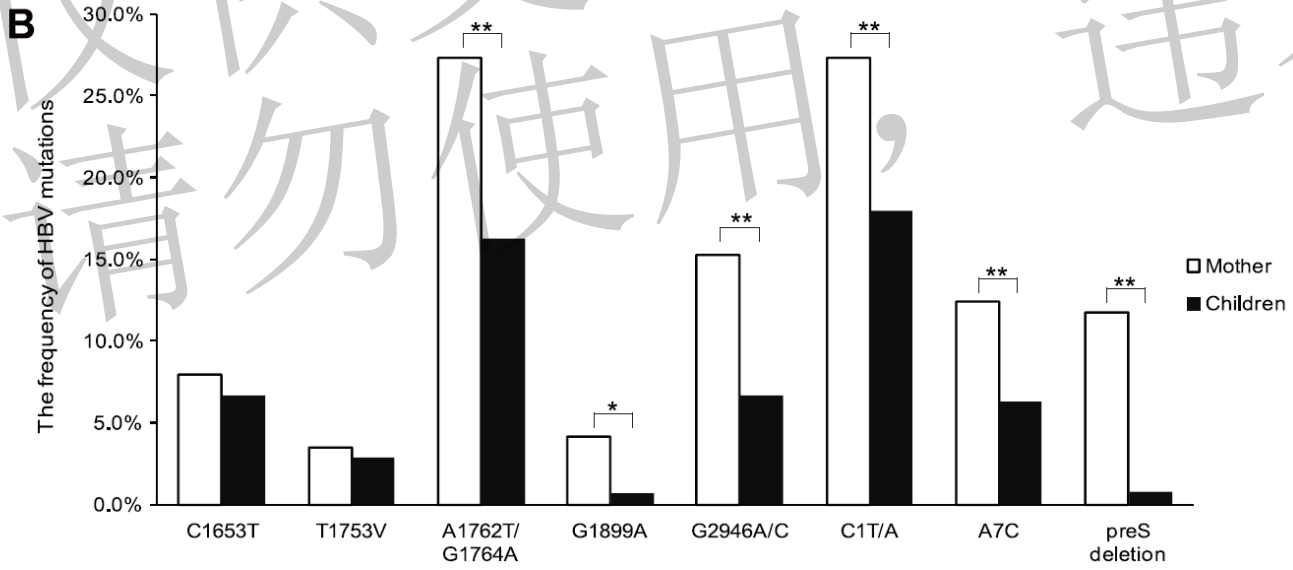
The frequencies of important HCC-risk HBV mutations in the mothers, matched cord blood, and their infants at 7 months of age.

HCC-risk mutations	Frequency, *n* (%)		
	Mother	Cord	Infant
Genotype B2			
C1653T	3 (2.4)	4 (4.0)	0
T1674C/G	2 (1.6)	1 (1.0)	0
A1762T/G1764A	7 (5.6)	12 (12.1)	1 (2.8)
G1799C	8 (6.4)	13 (13.1)	0
G1896A	7 (5.6)	0	0
C1T/A	12 (5.9)	8 (5.3)	0
preS1 start codon mutation	1 (0.5)	1 (0.7)	0
preS2 start codon mutation	3 (1.5)	4 (2.7)	0
preS deletion	17 (8.3)	15 (10)	1 (2.0)
Genotype C2			
C1653T	8 (7.8)	9 (12.7)	0
T1674C/G	43 (41.7)	34 (47.9)	0
T1753V	0	4 (5.6)	0
A1762T/G1764A	20 (19.4)	29 (40.8)	0
G1896A	5 (4.9)	0	0
G2946A	12 (7.5)	8 (5.7)	0
C1T/A	12 (7.5)	19 (13.6)	2 (3.8)
preS1 start codon mutation	3 (1.9)	1 (0.7)	0
preS2 start codon mutation	5 (3.1)	2 (1.4)	0
preS deletion	10 (6.2)	2 (1.4)	0

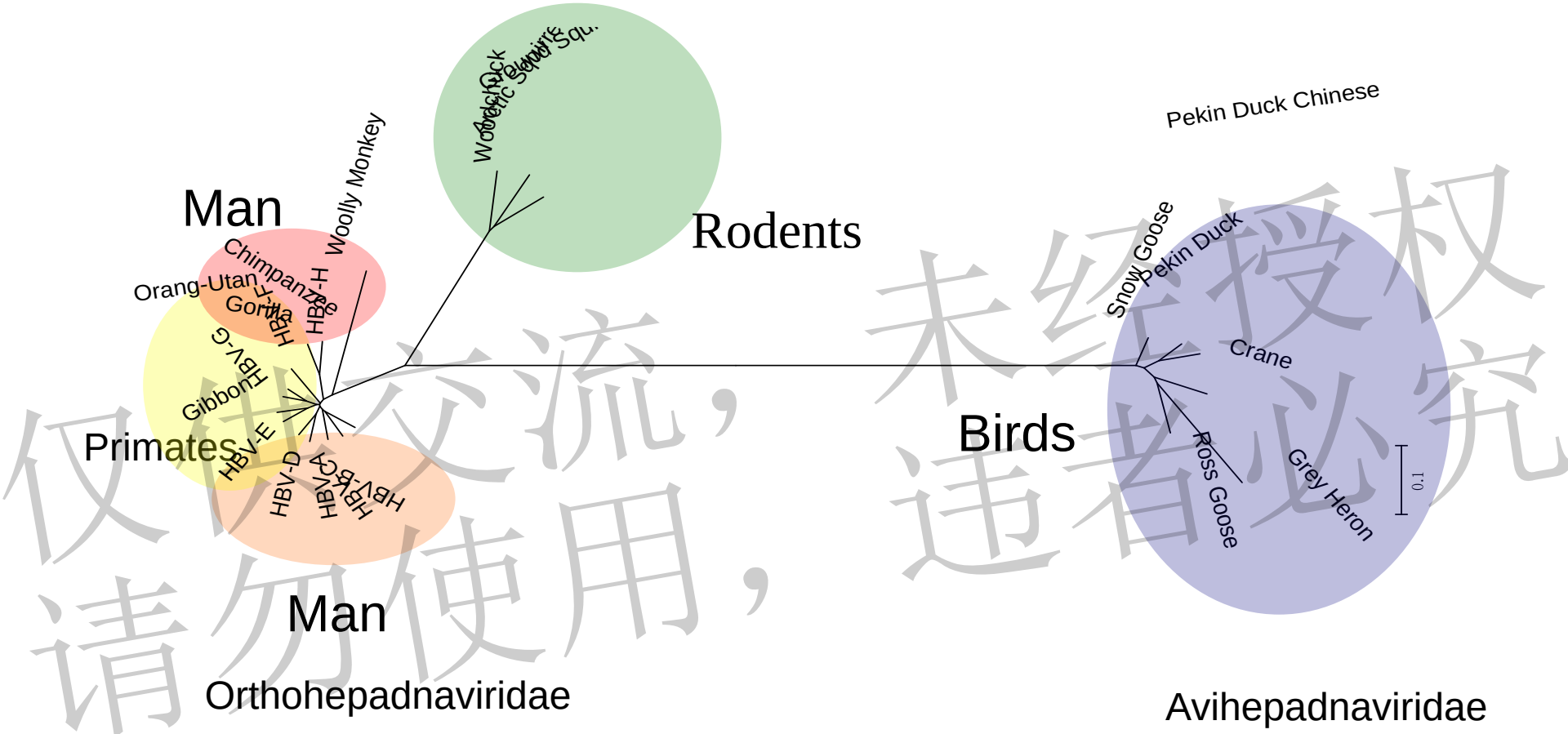
HBV, hepatitis B virus; HCC, hepatocellular carcinoma.

A1762T/G1764A is an earlier HCC-risk HBV mutation, C1653T and T1753V are later ones





J Clin Virol 2014;61:47-54



Evolution of hepadnaviridae among vertebrates

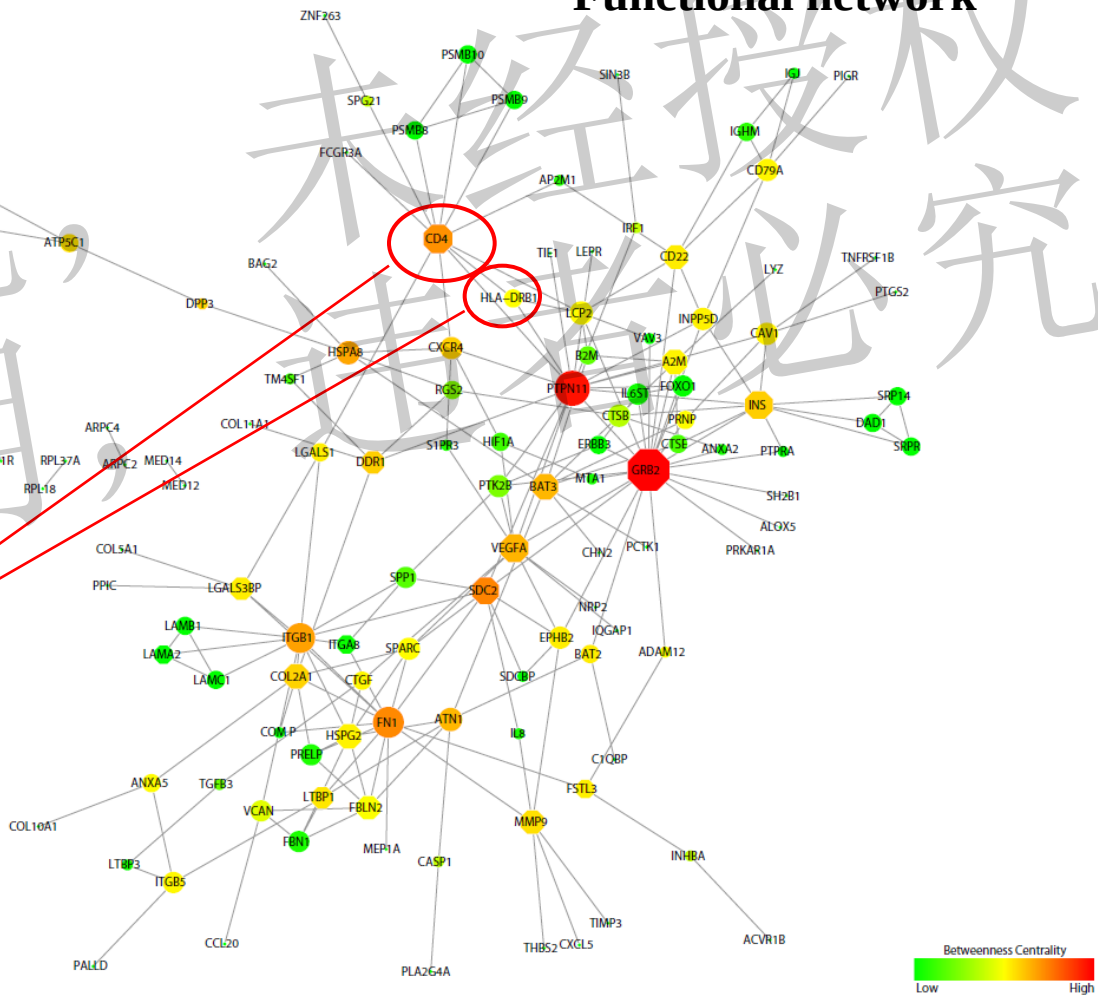
Pathways facilitating immune selection of the HCC-related HBV mutations

HBV mutation combo *vs.* none

PBMNC cDNA microarray

HLA Class II

Functional network



HLA-DP polymorphic genotypes promoting HBV persistence facilitate the immune selection of HCC-risk HBV mutations

TABLE 5 Significant associations of *HLA-DP* polymorphisms with frequencies of HBV mutations associates with liver disease risk[d]

	Genotype C HBV-infected subjects		Genotype B HBV-infected subjects	
HLA-DP SNP	HBV mutation	Adjusted OR (95% CI)	HBV mutation	Adjusted OR (95% CI)
rs3077				
CC		1.00		1.00
CT	C1653T	**0.68 (0.48–0.95)**		
	C1673T	**0.65 (0.43–0.99)**		
	Pre-S1 start codon mutation	**1.56 (1.02–2.39)**		
TT	A1846T	**0.54 (0.30–0.97)**	G1719T	**2.97 (1.06–8.37)**
	G1896A	**0.47 (0.27–0.80)**	G1730C	**2.63 (1.01–6.85)**
	C10A[a]	**0.40 (0.17–0.94)**		
	Pre-S deletion	**0.43 (0.20–0.91)**		
rs3135021				
GG		1.00		1.00
GA	C1673T[b]	**0.54 (0.35–0.84)**		
	Pre-S1 start codon mutation	**2.04 (1.34–3.11)**		
AA	A1727T	**2.95 (1.20–7.23)**		
	A1846T	**0.47 (0.23–0.95)**		
rs9277535				
GG		1.00		1.00
GA				
AA	T1674C/G	**0.62 (0.39–0.97)**		
	A1846T	**0.62 (0.39–0.98)**		
rs2281388				
CC		1.00		1.00
CT	T1674C/G	**1.47 (1.04–2.06)**	T1674C	**0.35 (0.14–0.87)**
	Pre-S deletion	**1.49 (1.02–2.17)**	G1719T	**0.41 (0.20–0.84)**
	Pre-S2 start codon mutation	**1.54 (1.02–2.33)**	G1652A[c]	**0.46 (0.24–0.91)**
			A1C	**0.33 (0.13–0.82)**
TT	A1846T	**1.61 (1.06–2.44)**	G1719T	**0.41 (0.18–0.93)**
	G1896A	**1.63 (1.11–2.42)**		
	Pre-S2 start codon mutation	**1.99 (1.23–3.24)**		
	Pre-S deletion	**1.90 (1.21–2.98)**		

[a] Represents highly correlated (phi coefficient, >0.7) mutations, including A31T, T49A, G105C, C109A, A135C, and G147C.
[b] Represents highly correlated (phi coefficient, >0.7) mutations, including A1652G, C1730G, and C1799G.
[c] Represents highly correlated (phi coefficient, > 0.7) mutations, including T1673C, G1730C, and G1799C.

J Virol **2013**;87(22):12176-86.

HBV mutation increase the risk of HCC solely in the patients with specific HLA-DP polymorphic genotypes

TABLE 7 Associations of the interaction of *HLA* SNPs and HBV mutations with the risk of HCC in genotype C HBV-infected subjects[a]

HLA-DP SNP	HBV mutation	No. of HCC-free HBV-infected subjects	No. of HCC patients	Adjusted OR (95% CI)	*P* value
rs9277535	T1674C/G				
GG	T	118	92	1.00	
GG	C/G	52	75	**1.64 (1.02–2.62)**	**0.039**
AA	T	46	43	1.12 (0.66–1.90)	0.682
AA	C/G	24	14	0.69 (0.46–1.03)	0.067
For interaction				**0.35 (0.14–0.88)**	**0.026**
rs9277535	G1719T				
GG	G	90	37	1.00	
GG	T	94	130	**3.13 (1.94–5.06)**	**<0.001**
AA	G	30	20	1.42 (0.68–3.00)	0.354
AA	T	46	37	**1.40 (1.02–1.91)**	**0.037**
For interaction				**0.40 (0.17–0.97)**	**0.042**

[a] Boldface type indicates significant values.

J Virol **2013**;87(22):12176-86

STAT3 polymorphic genotypes increasing HCC risk facilitate the immune selection of HCC-risk HBV mutations

Table 3. Associations of STAT3 SNPs With Frequencies of T1674C/G and A1762T/G1764A Using Data of HBV-Infected Patients, Including HCC Patients

		T1674C/G			A1762T/G1764A		
SNP (Accession Code)	Genotype/Allele	T	C/G	AOR (95% CI)	AG/AA/TG	TA	AOR (95% CI)
rs4796793 (C>G)	CC	322	111	1.00	169	287	1.00
	CG	328	105	0.92 (0.67-1.25)	191	275	0.83 (0.63-1.09)
	GG	105	34	1.00 (0.64-1.57)	50	91	1.11 (0.74-1.66)
	G (CG+GG)	433	139	0.93 (0.70-1.24)	241	366	0.88 (0.68-1.14)
rs2293152 (C>G)	CC	208	51	1.00	127	153	1.00
	CG	341	129	**1.49 (1.03-2.16)**	182	316	**1.41 (1.04-1.91)**
	GG	171	66	**1.61 (1.06-2.46)**	88	160	**1.64 (1.14-2.35)**
	G (CG+GG)	512	195	**1.53 (1.08-2.17)**	270	476	**1.48 (1.11-1.97)**
rs1053004 (T>C)	TT	305	108	1.00	162	274	1.00
	TC	340	109	0.91 (0.67-1.24)	193	287	0.88 (0.67-1.16)
	CC	106	27	0.79 (0.48-1.28)	53	84	0.98 (0.65-1.48)
	C (TC+CC)	446	136	0.88 (0.66-1.18)	246	371	0.90 (0.69-1.16)

Hepatology 2013;57(6):2369-2377.

Table 4. Contributions of STAT3 SNPs and Their Interactions With HBV Mutations in the EnhII/BCP/PC Region to HCC Risk

Variables	AOR (95% CI)	*P*
rs4796793 (C>G)		
Age, years	**1.03 (1.01-1.05)**	**0.001**
Sex, male versus female	**1.81 (1.08-3.05)**	**0.025**
Viral load, $\geq 10^4$ versus $< 10^4$ copies/mL	0.88 (0.57-1.36)	0.567
T1674C/G	**1.90 (1.09-3.31)**	**0.024**
A1762T/G1764A	1.32 (0.80-2.15)	0.275
A1726C	1.40 (0.77-2.56)	0.268
rs4796793, GG versus CC	0.90 (0.51-1.59)	0.707
rs4796793, (GG versus CC) × T1674C/G	0.97 (0.31-3.05)	0.962
rs2293152 (C>G)		
Age, years	**1.04 (1.02-1.06)**	**2.94E-04**
Sex, male versus female	**1.86 (1.06-3.26)**	**0.031**
Viral load, $\geq 10^4$ versus $< 10^4$ copies/mL	1.34 (0.83-2.16)	0.232
T1674C/G	**2.06 (1.14-3.70)**	**0.016**
A1762T/G1764A	**2.10 (1.24-3.56)**	**0.006**
A1726C	**2.39 (1.00-5.69)**	**0.050**
rs2293152, GG versus CC	**1.98 (1.18-3.34)**	**0.010**
rs2293152, (GG versus CC) × A1726C	**0.20 (0.06-0.69)**	**0.011**
rs1053004 (T>C)		
Age, years	**1.04 (1.02-1.05)**	**4.47E-06**
Sex, male versus female	**1.92 (1.27-2.92)**	**0.002**
Viral load, $\geq 10^4$ versus $< 10^4$ copies/mL	1.11 (0.78-1.58)	0.570
T1674C/G	1.33 (0.77-2.29)	0.315
A1762T/G1764A	1.45 (0.98-2.16)	0.065
A1726C	1.28 (0.78-2.10)	0.337
rs1053004, TC versus TT	0.71 (0.47-1.06)	0.093
rs1053004, (TC versus TT) × T1674C/G	**2.25 (1.02-4.95)**	**0.044**

Interaction of STAT3 polymorphic genotypes and HBV mutations increase the risk of HCC

Hepatology **2013**;57(6):2369-2377.

NF-κB polymorphic genotypes increasing HCC risk facilitate the immune selection of HCC-risk HBV mutations

Table 3. Significant associations of the NF-κB polymorphisms with the frequencies of the HCC-risk HBV mutations

NF-κB SNP	Genotype C HBV-infected subjects		Genotype B HBV-infected subjects	
	HBV mutation	AOR (95% CI)	HBV mutation	AOR (95% CI)
rs2233406				
CC		1		1
CT	T1753V	2.09 (1.47–2.99)	–	
	A1762T/G1764A	1.55 (1.08–2.22)		
	preS deletion	1.42 (1.00–2.01)		
TT	G1719T	4.53 (1.02–20.05)	–	
	preS1 start codon mutation	3.13 (1.17–8.38)		
CT + TT	T1753V	1.92 (1.36–2.72)	–	
	A1762T/G1764A	1.49 (1.05–2.11)		
	preS1 start codon mutation	1.54 (1.00–2.38)		
rs28362491				
Ins/Ins		1		1
Ins/Del	preS2 start codon mutation	0.60 (0.42–0.85)	–	
Del/Del	A1762T/G1764A	1.62 (1.07–2.46)	G1899A	3.12 (1.01–9.60)
	preS2 start codon mutation	0.58 (0.35–0.94)		
Ins/Del + Del/Del	A1762T/G1764A	1.35 (1.01–1.81)	–	
	preS2 start codon mutation	0.59 (0.43–0.83)		

AOR, adjusted odds ratio (adjusted for age and gender); CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; SNP, single-nucleotide polymorphism.

Ann Oncol **2014**;25(12):2413-9.

HBV mutation increase the risk of HCC only in the patients with certain NF-κB polymorphic genotypes

Table 4. Interactions of NF-κB polymorphisms with the HBV mutations on the risk of HCC in the genotype C HBV-infected subjects

NF-κB SNP	HBV mutations	HCC-free HBV-infected subjects	HBV–HCC	AOR (95% CI)	*P* value
rs2233406	A1762T/G1764A				
CC	AG/AA/TG	216	73	1	
CC	TA	174	223	3.52 (2.50–4.95)	4.21×10^{-13}
CT + TT	AG/AA/TG	51	9	0.50 (0.23–1.07)	0.073
CT + TT	TA	46	85	2.16 (1.72–2.72)	4.10×10^{-11}
For interaction				2.61 (1.09–6.26)	0.032
rs2233406	preS2 start codon mutation				
CC	0	289	213	1	
CC	1	55	96	2.23 (1.52–3.28)	4.45×10^{-5}
CT + TT	0	85	89	1.44 (1.00–2.06)	0.048
CT + TT	1	20	22	1.14 (0.82–1.58)	0.429
For interaction				0.42 (0.19–0.93)	0.032

AOR, adjusted odds ratio (adjusted for age and gender); CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; SNP, single-nucleotide polymorphism.

Ann Oncol **2014**;25(12):2413-9.

miRNAs involved in HBV activities

Table 1
The host-derived miRNAs affect the replication and gene expression of HBV.

miRNAs	Potential target sites/molecules	Functions
let-7	HBV S gene; HBV DNA polymerase	NA
miR-1	Host genes (FXRA, HDAC4, E2F5)	Increase HBV antigen expression and DNA replication
miR-29c	Host gene (TNFAIP3)	Suppress HBV antigen expression and DNA replication
miR-122	HBV DNA polymerase; 3′-UTR of the HBV core gene; Host genes (HO-1, cyclin G1)	Suppress HBsAg expression and DNA replication
miR-125a-5p	HBV S gene; HBV DNA polymerase	Suppress HBsAg translation
miR-155	Host gene (SOCS1)	Suppress HBV X expression
miR-196b	HBV S gene; HBV DNA polymerase	NA
miR-199a-3p	HBV S gene; HBV DNA polymerase	Suppress HBsAg expression and DNA replication
miR-205	HBV X gene	NA
miR-210	HBV pre-S1 gene; HBV DNA polymerase	Suppress HBsAg expression and DNA replication
miR-345	HBV preC gene	NA
miR-372/373	Host gene (nuclear factor I/B)	Increase HBV proteins and HBV core-associated DNA
miR-433	HBV S gene; HBV DNA polymerase	NA
miR-511	HBV S gene; HBV DNA polymerase	NA

Cancer Lett 2012(321): 1–12.

miRs affecting HBV mutations: miR-218, miR-34b/c

miR-218 polymorphic genotypes increasing HCC risk facilitate the immune selection of HCC-risk HBV preS deletion in male HBV-infected subjects

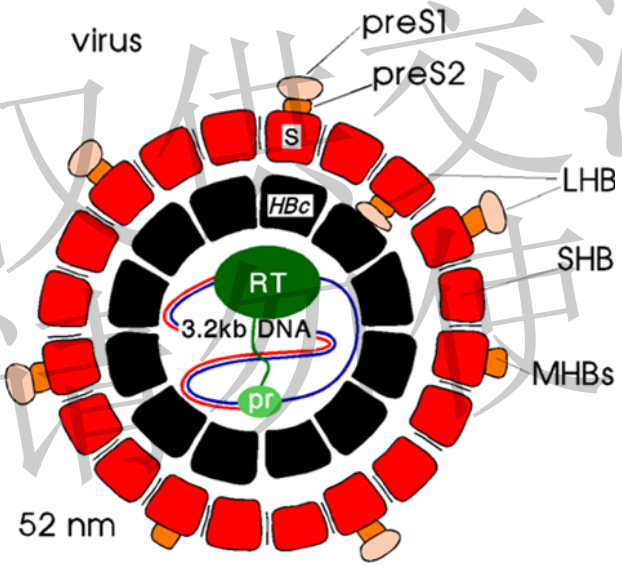


Table 5. Association of the *pre-miR-218* rs11134527 variant genotypes with the generation of HBV preS deletion in the HBsAg-positive subjects including the HCC patients

		HBV preS deletion			
Gender group	rs11134527	No	Yes	Adjusted OR (95% CI)*	*P*-value
Combined	AA	226	56	1.00	
	AG	243	113	1.35 (1.12–1.62)	0.002
	GG	109	43	1.54 (0.97–2.44)	0.066
	G (GG + AG)	352	156	1.72 (1.21–2.44)	0.003
Women	AA	66	14	1.00	
	AG	66	21	1.22 (0.84–1.79)	0.303
	GG	26	6	1.07 (0.37–3.12)	0.896
	G (GG + AG)	92	27	1.35 (0.66–2.79)	0.413
Men	AA	160	42	1.00	
	AG	177	92	1.39 (1.12–1.72)	0.003
	GG	83	37	1.70 (1.01–2.84)	0.046
	G (GG + AG)	260	129	1.85 (1.23–2.76)	0.003

Liver Int 2014:34(5):728-736

miR-218 polymorphic genotypes increasing HCC risk facilitate the immune selection of HCC-risk HBV preS deletion in male HBV-infected subjects

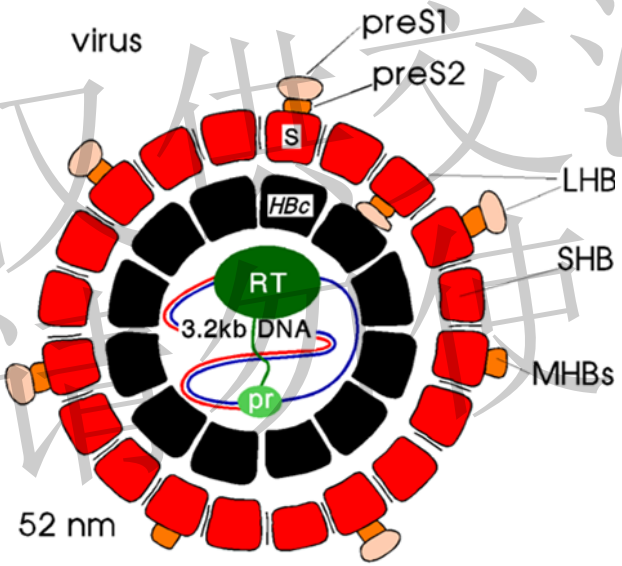


Table 5. Association of the *pre-miR-218* rs11134527 variant genotypes with the generation of HBV preS deletion in the HBsAg-positive subjects including the HCC patients

Gender group	rs11134527	HBV preS deletion No	Yes	Adjusted OR (95% CI)*	*P*-value
Combined	AA	226	56	1.00	
	AG	243	113	1.35 (1.12–1.62)	0.002
	GG	109	43	1.54 (0.97–2.44)	0.066
	G (GG + AG)	352	156	1.72 (1.21–2.44)	0.003
Women	AA	66	14	1.00	
	AG	66	21	1.22 (0.84–1.79)	0.303
	GG	26	6	1.07 (0.37–3.12)	0.896
	G (GG + AG)	92	27	1.35 (0.66–2.79)	0.413
Men	AA	160	42	1.00	
	AG	177	92	1.39 (1.12–1.72)	0.003
	GG	83	37	1.70 (1.01–2.84)	0.046
	G (GG + AG)	260	129	1.85 (1.23–2.76)	0.003

***Liver Int* 2014:34(5):728-736**

miR-34b/c polymorphic genotypes increasing HCC risk facilitate the immune selection of HCC-risk HBV T1674C/G mutation

Table 3. The associations of the polymorphism with the HCC-related HBV mutations using the data of all HBV-infected subjects

SNP	Genotype/allele	T1674C/G				G1896A			
		T(%)	C/G(%)	AOR (95% CI)	*P* value	G (%)	A (%)	AOR (95% CI)	*P* value
Pri-miR-34b/c (rs4938723, T >C)	TT	358(47.3)	102(40.8)	1.00		282(44.2)	191(46.9)	1.00	
	TC	322(42.5)	108(43.2)	1.09(0.93–1.28)	0.269	285(44.7)	167(41.0)	0.93(0.81–1.06)	0.278
	CC	77(10.2)	40(16.0)	1.77(1.13–2.76)	0.012	71(11.1)	49(12.0)	0.99(0.65–1.50)	0.959
	C(TC+CC)	399(52.7)	148(59.2)	1.31(0.98–1.75)	0.073	356(55.8)	216(53.1)	0.89(0.69–1.14)	0.358
Pre-miR-196a2 (rs11614913, T >C)	TT	205(27.0)	75(29.9)	1.00		197(30.8)	109(26.8)	1.00	
	TC	379(50.0)	119(47.4)	0.93(0.79–1.10)	0.403	291(45.5)	222(54.5)	1.17(1.01–1.36)	0.035
	CC	174(23.0)	57(22.7)	0.87(0.58–1.30)	0.487	152(23.8)	76(18.7)	0.84(0.58–1.22)	0.353
	C(TC+CC)	553(73.0)	176(70.1)	0.87(0.63–1.19)	0.370	443(69.2)	298(73.2)	1.19(0.90–1.58)	0.216

Genetic predispositions of genes in the key inflammatory pathways determine the selection of HBV mutations

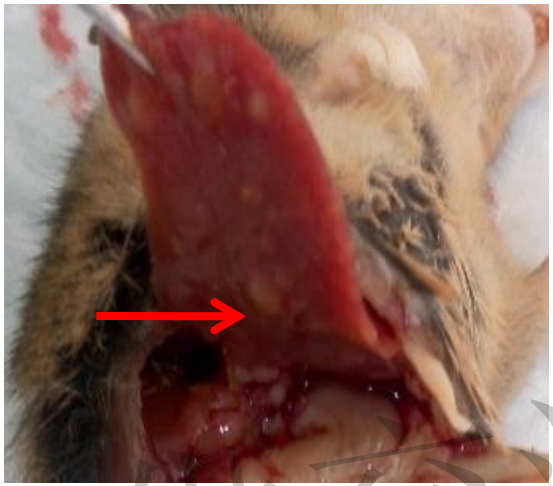
① The genetic predipositions of STAT3, NF-κB, HLA-II, and miR-218 coding regions are associated with chronic HBV infection and immune selection of HCC-promoting HBV mutations;

② SNP genotypes that facilitating the generation of the HBV mutations are dominant in Chinese, not in European

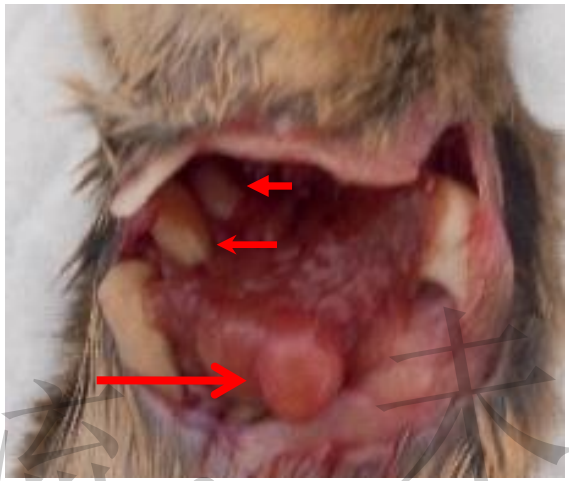
Dominant allele[a], *n* (%)		
Han Chinese	European	
A (82.6)	G (50.0)	NFKBIA
A (53.3)	G (73.3)	HLA-DQ
G (88.4)	G (52.2)	HLA-DQ
G (81.4) for Japanese		HLA-DQ
A (81.4)	G (50.0)	HLA-DQ
G (62.5)	A (88.3)	HLA-DQ
G (61.9)	A (83.2)	HLA-DP
A (61.4)	G (88.3)	HLA-DP
C (75.6)	T (73.2)	HLA-DP
G (59.8)	G (94.2)	HLA-DP
G (55.2)	T (69.2)	HLA-DP
G (58.3)	G (74.8)	HLA-DP
C (68.6)	C (92.5)	HLA-DP

***Hepatology* 2013; *Ann Oncol* 2014; *J Virol* 2013; *Liver Int* 2014; *EMI* 2015**

HBx mutants promote HCC in sleeping beauty model

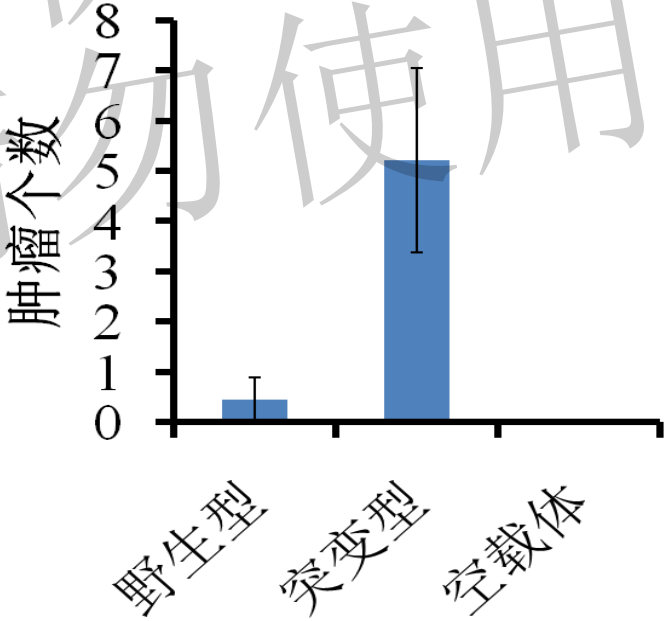


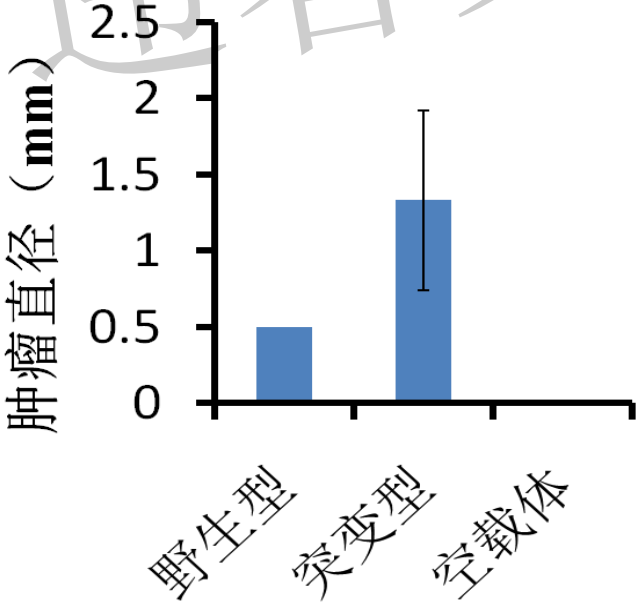
野生型HBx



突变型HBx

空载体

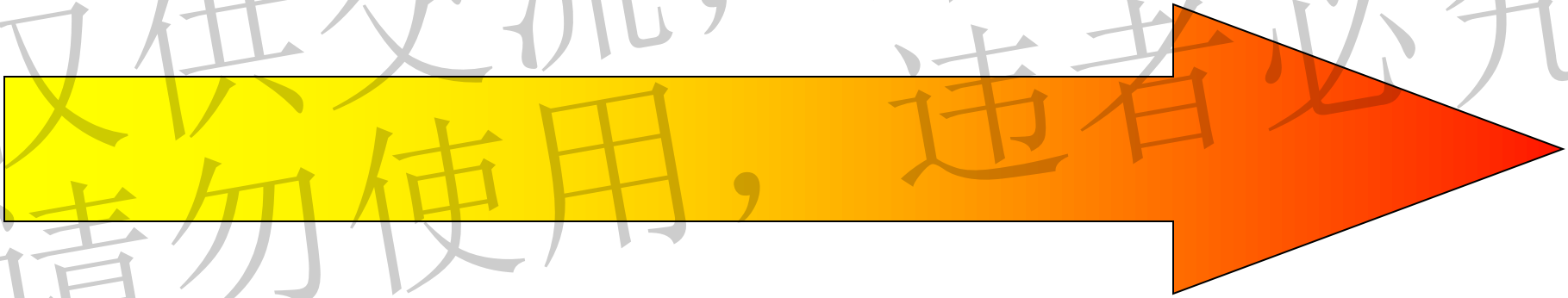




Knowledge continuum

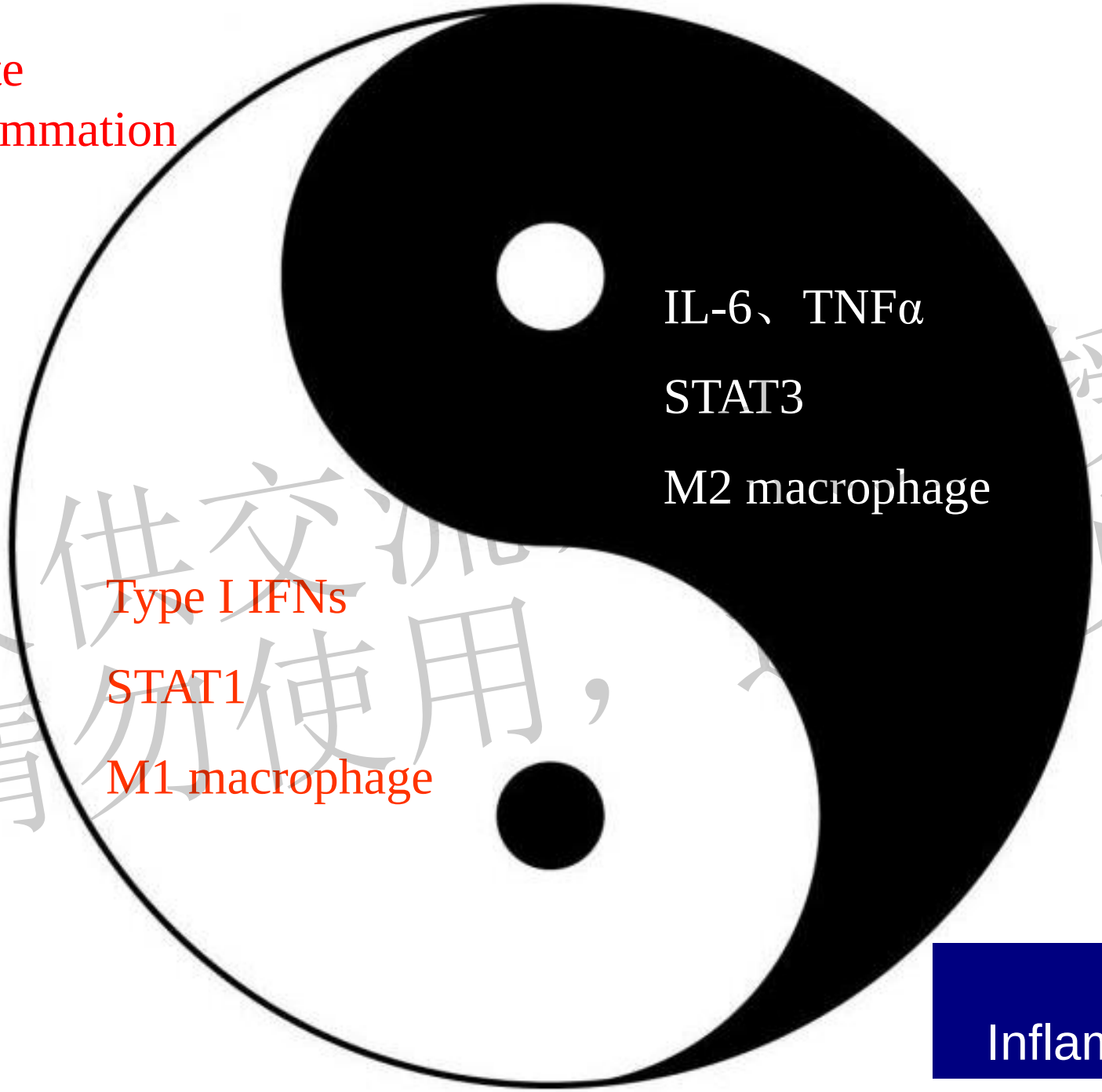
Less information
Looking for clues

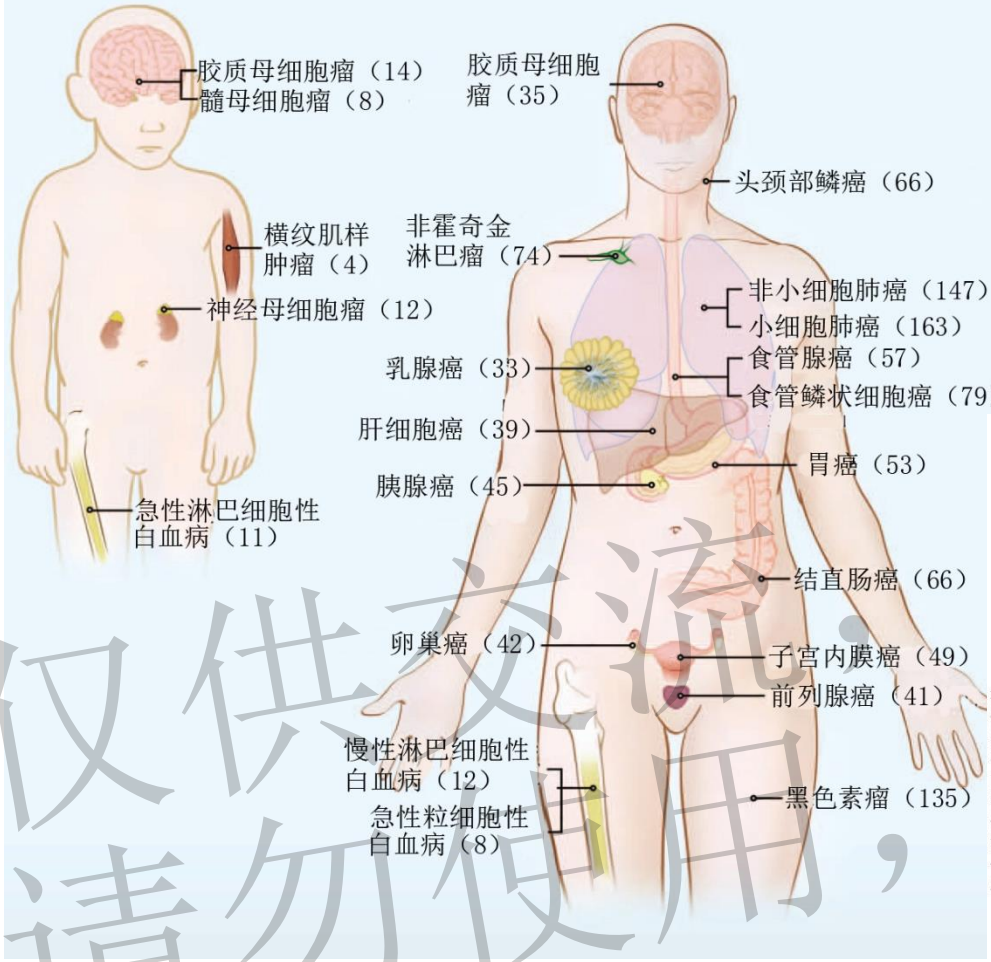
More information
Identifying causal relationships



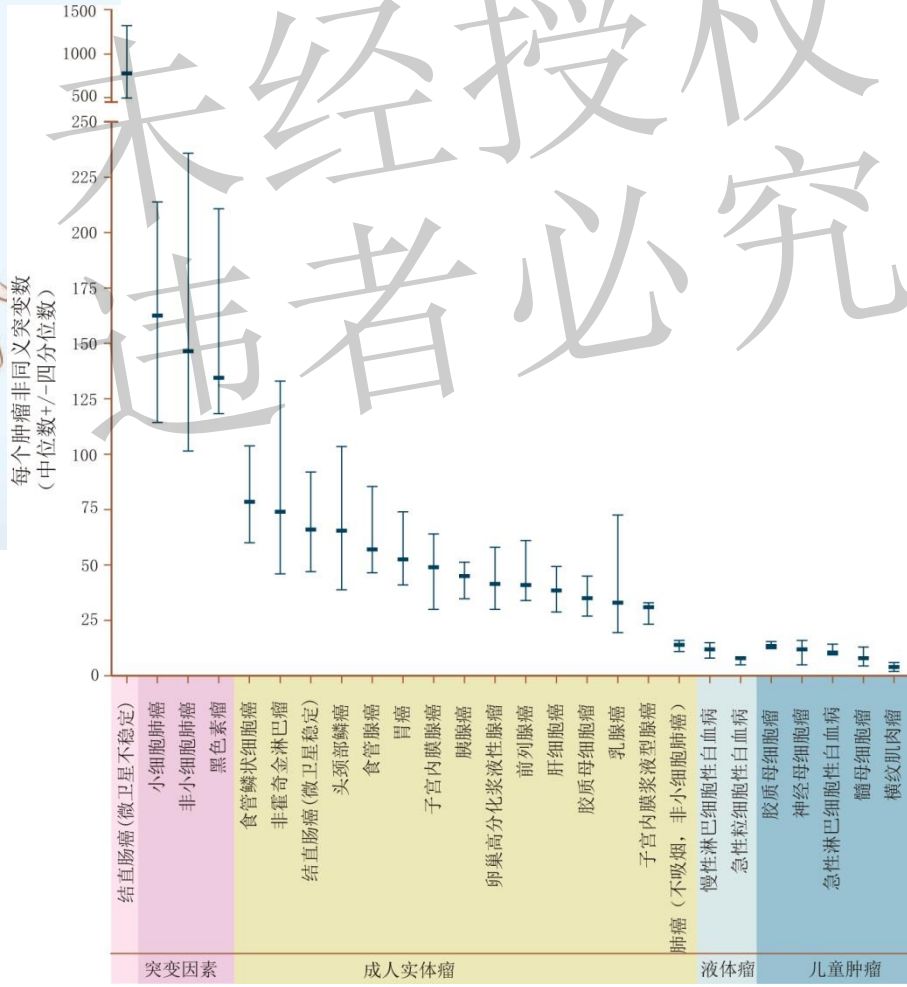
Descriptive studies

Analytical studies





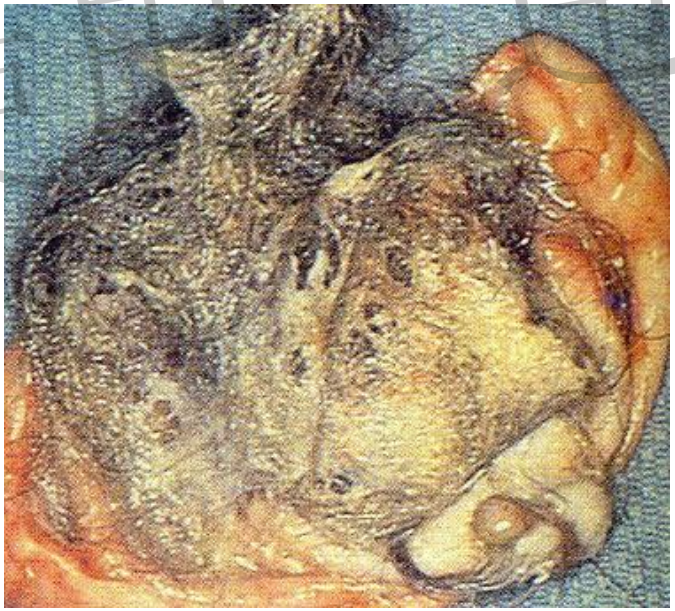
HCC中只有39个非同义突变



全基因组测序确定成人肿瘤和儿童肿瘤体细胞非同义变异数（每个肿瘤携带非同义变异平均数）

Inspiration of *Cancer Evo-Dev* derived from 4 stories

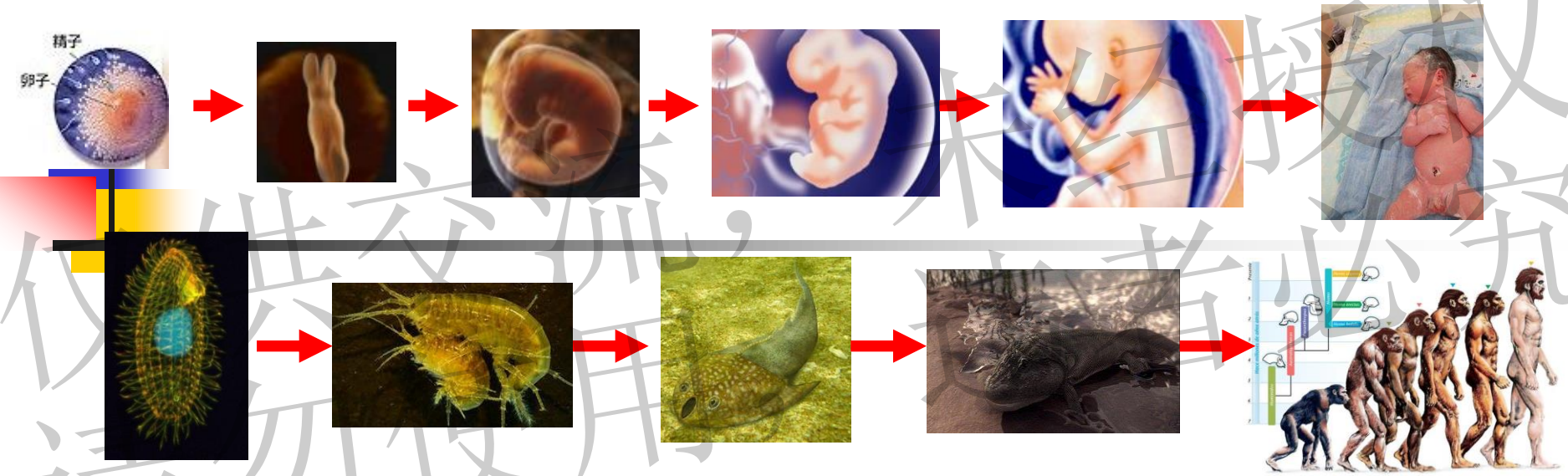
1. When I was a medical student, a malignancy termed as malignant teratoma drew my attention because it contains a lot of different kinds of embryo tissues…..



2. Genes whose ORFs are open during fetal period and silenced after birth are frequently associated with cancers

- HCC biomarkers
  - Alpha-fetoprotein (AFP): hepatocellular carcinoma, 70%
  - SALL4, HCC
- Gene signature active in embryonic development are also related to cancer progression and metastasis

3. The process of embryonic development share the similarity with the process of life evolution



- The genes such as *Hox*, *Hedgehog*, *Myc* linking the development and evolutionary processes

How these observations related to cancers

- Cancers are events of retro-differentiation, back to the progenitors;
- Cancers are genetic diseases whose genetics are in retro-evolutionary orders;
- Cancer initiation cells evolved from chronic inflammation that provides suitable conditions for retro-differentiation

Somatic Mutations, Viral Integration and Epigenetic Modification in the Evolution of Hepatitis B Virus-Induced Hepatocellular Carcinoma

Xiaowei Ji[+], Qi Zhang[+], Yan Du, Wenbin Liu, Zixiong Li, Xiaomei Hou and Guangwen Cao*[,#]

Department of Epidemiology, Second Military Medical University, Shanghai 200433, China

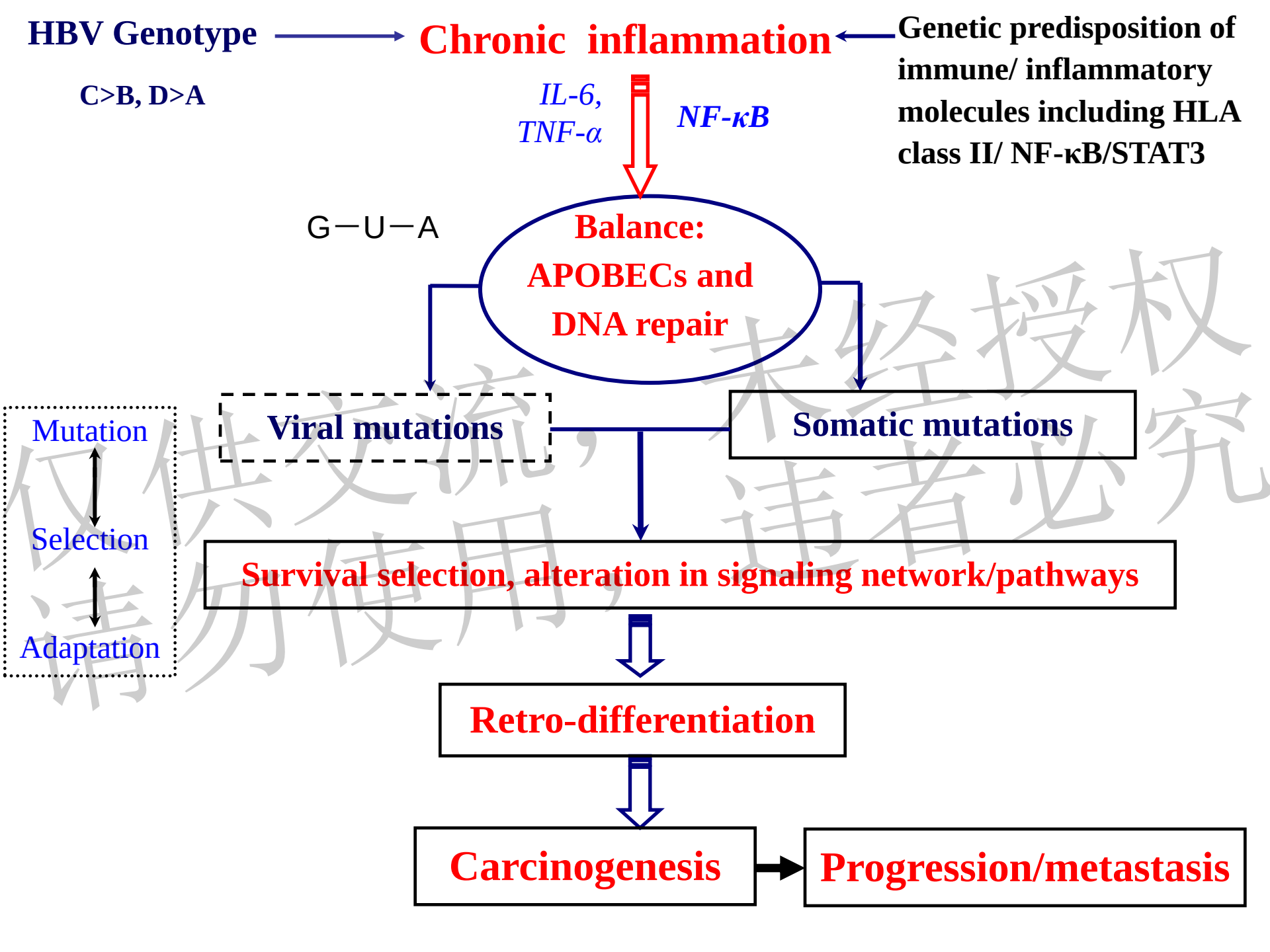
We presented a theory framework of "*Cancer Evo-Dev*" systemically

Abstract: Liver cancer in men is the second leading cause of cancer death and hepatocellular carcinoma (HCC) accounts for 70%-85% of the total liver cancer worldwide. Chronic infection with hepatitis B virus (HBV) is the major cause of HCC. Chronic, intermittently active inflammation provides "fertile field" for "mutation, selection, and adaptation" of HBV and the infected hepatocytes, a long-term evolutionary process during HBV-induced carcinogenesis. HBV mutations, which are positively selected by insufficient immunity, can promote and predict the occurrence of HCC. Recently, advanced sequencing technologies including whole genome sequencing, exome sequencing, and RNA sequencing provide opportunities to better understand the insight of how somatic mutations, structure variations, HBV integrations, and epigenetic modifications contribute to HCC development. Genomic variations of HCC caused by various etiological factors may be different, but the common driver mutations are important to elucidate the HCC evolutionary process. Genome-wide analyses of HBV integrations are helpful in clarifying the targeted genes of HBV in carcinogenesis and disease progression. RNA sequencing can identify key molecules whose expressions are epigenetically modified during HCC evolution. In this review, we summarized the current findings of next generation sequencings for HBV-HCC and proposed a theory framework of *Cancer Evolution and Development* based on the current knowledge of HBV-induced HCC to characterize and interpret evolutionary mechanisms of HCC and possible other cancers. Understanding the key viral and genomic variations involved in HCC evolution is essential for generating effective diagnostic, prognostic, and predictive biomarkers as well as therapeutic targets for the interventions of HBV-HCC.

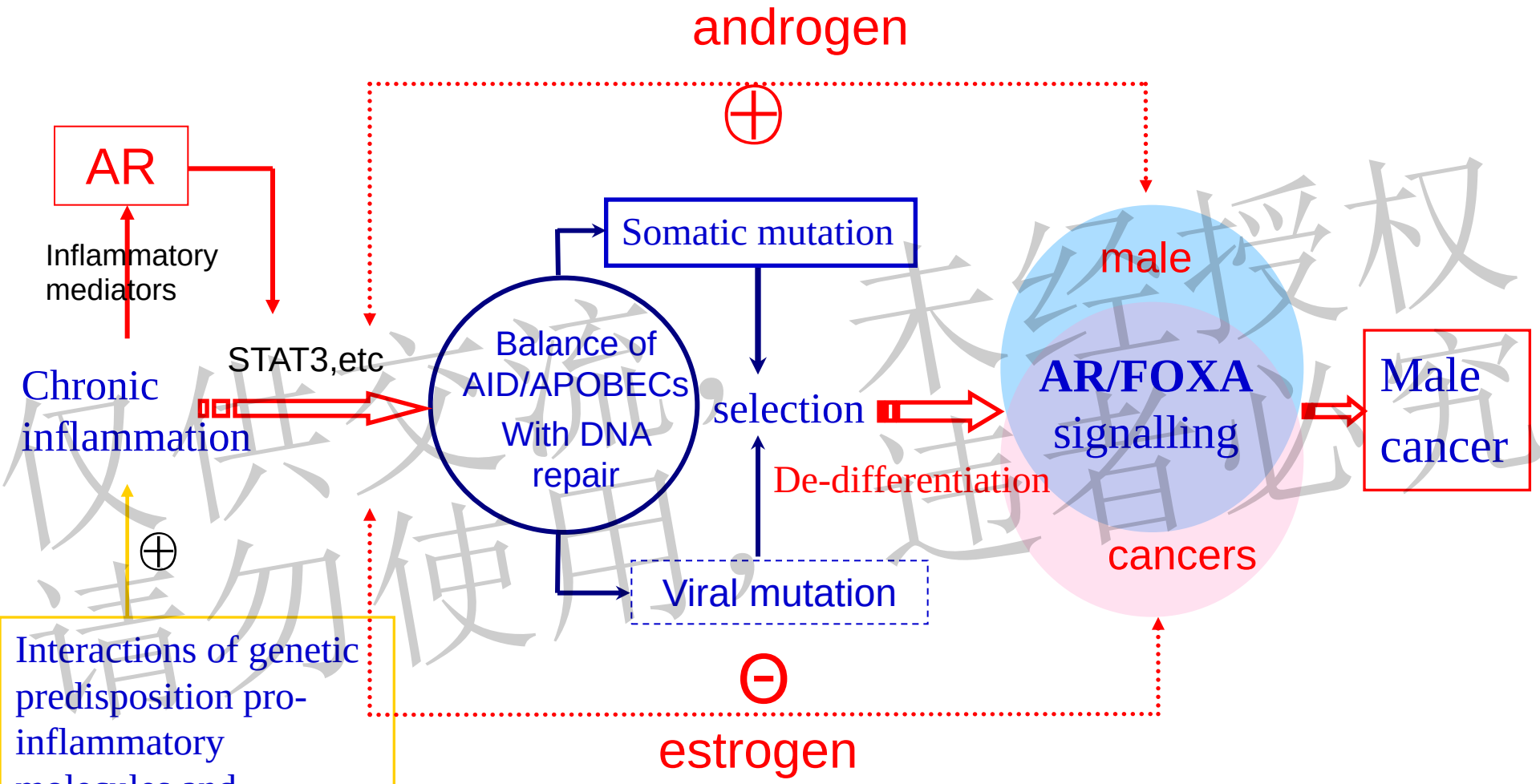
Received on: August 11, 2014- Revised on: November 11, 2014- Accepted on: November 14, 2014

Keywords: Hepatitis B virus, Hepatocellular carcinoma, Somatic mutation, Integration, Epigenetic modification, Deep sequencing.

[#]**Author's Profile:** Prof. Guangwen Cao is the chairman of the Department of Epidemiology, Second Military Medical University (Shanghai, China). He is the principal scientist of the National Key Basic Research Program (973 Program) in cancer research and the Distinguished Young Scholar in China. Prof. Cao has published 110 research papers in international journals.



Evolution and development of male-predominant cancers



Evolutionary procedure “mutation--selection--adaptation”

（***Hepatology* 2013; *Curr Genomics* 2014; *Cancer Lett* 2014; *J Virol* 2013; *Nat Genet* 2013**）

Types of analytical studies

- Observational studies
  - Cohort studies
  - Case-control studies
- Experimental studies

Cohort studies to answer 3 scientific questions

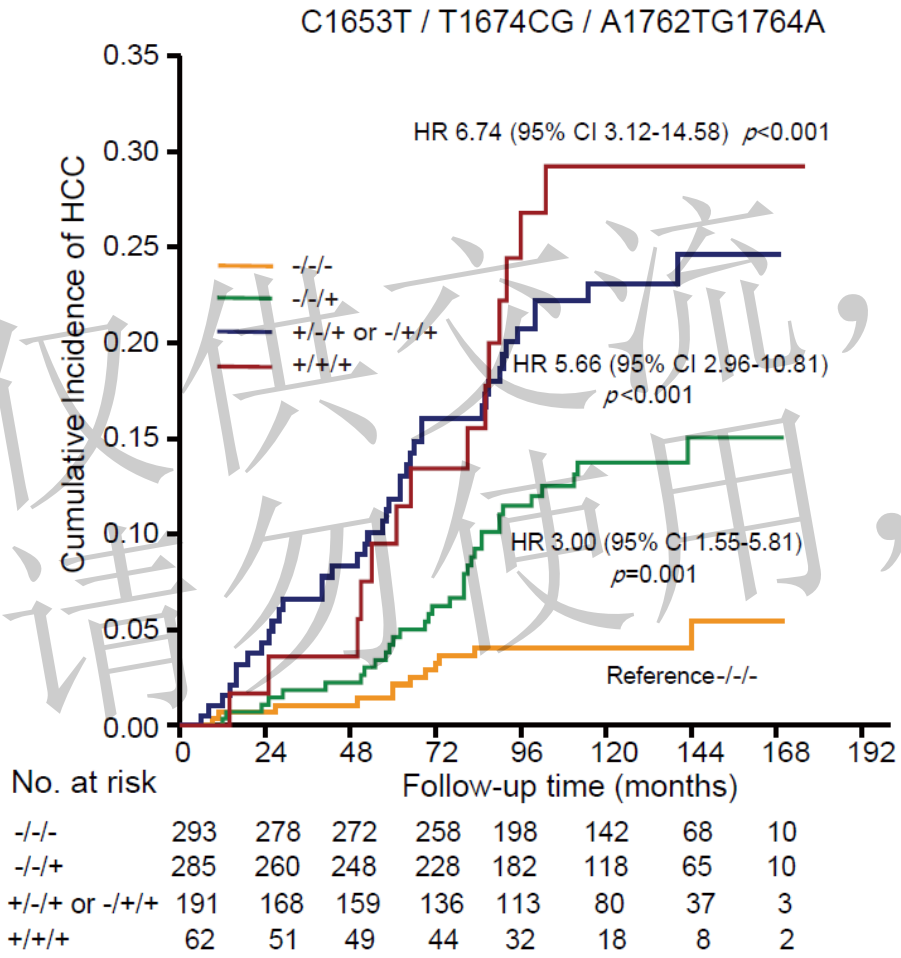
- **To identify HBV-infected subjects (or patients) who are more likely to develop HCC?**
- **To identify HBV-HCC patients who survive shortly after curative surgery?**
- **To elucidate the prophylactic methods can be effective in reducing HCC occurrence and recurrence**

Cohort 1: HBV-induced carcinogenesis

- 2114 of 2512 HCC-free, decompensated cirrhosis-free, chronic HBV-infected subjects;
- Of those 614 received ≥48 wks' standard antiviral treatment using NAs and or IFN-α;
- Followed-up for 18406 person-years;
- Propensity score matching was applied to reduce baseline differences between antiviral and control cohorts.

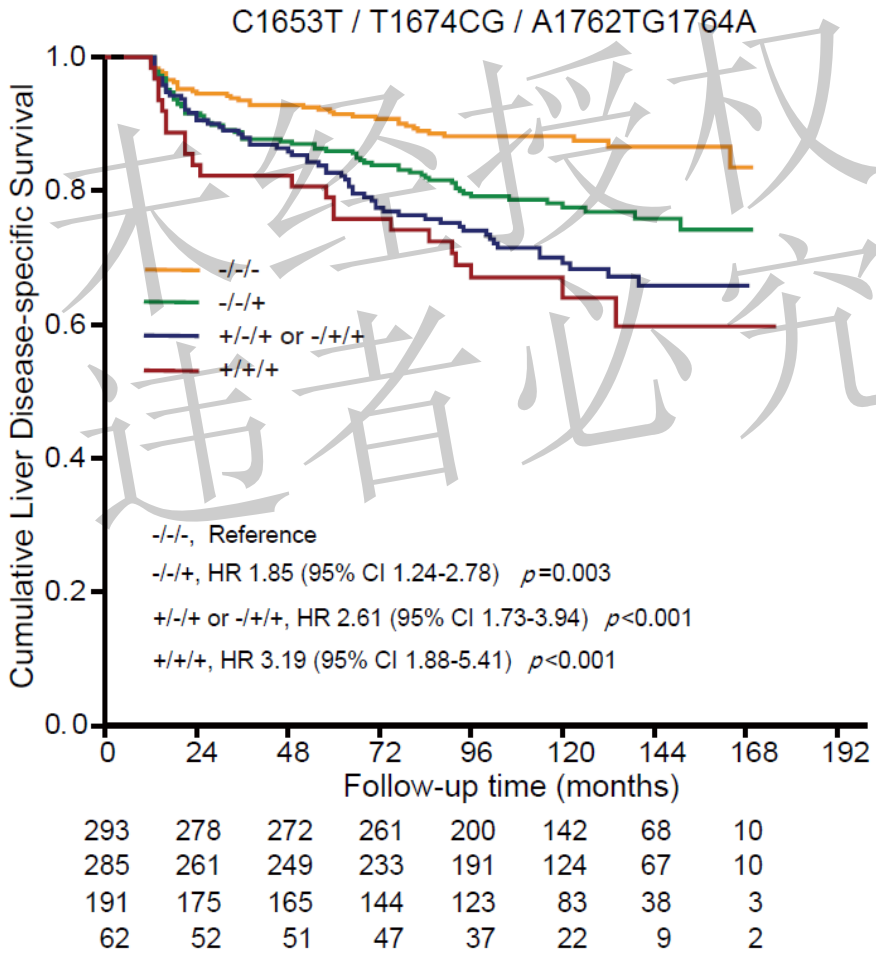
***Cancer Prev Res (Phila)* 2015;8(10):978-88**

HBV combo mutations at baseline effectively predict HCC occurrence and liver death

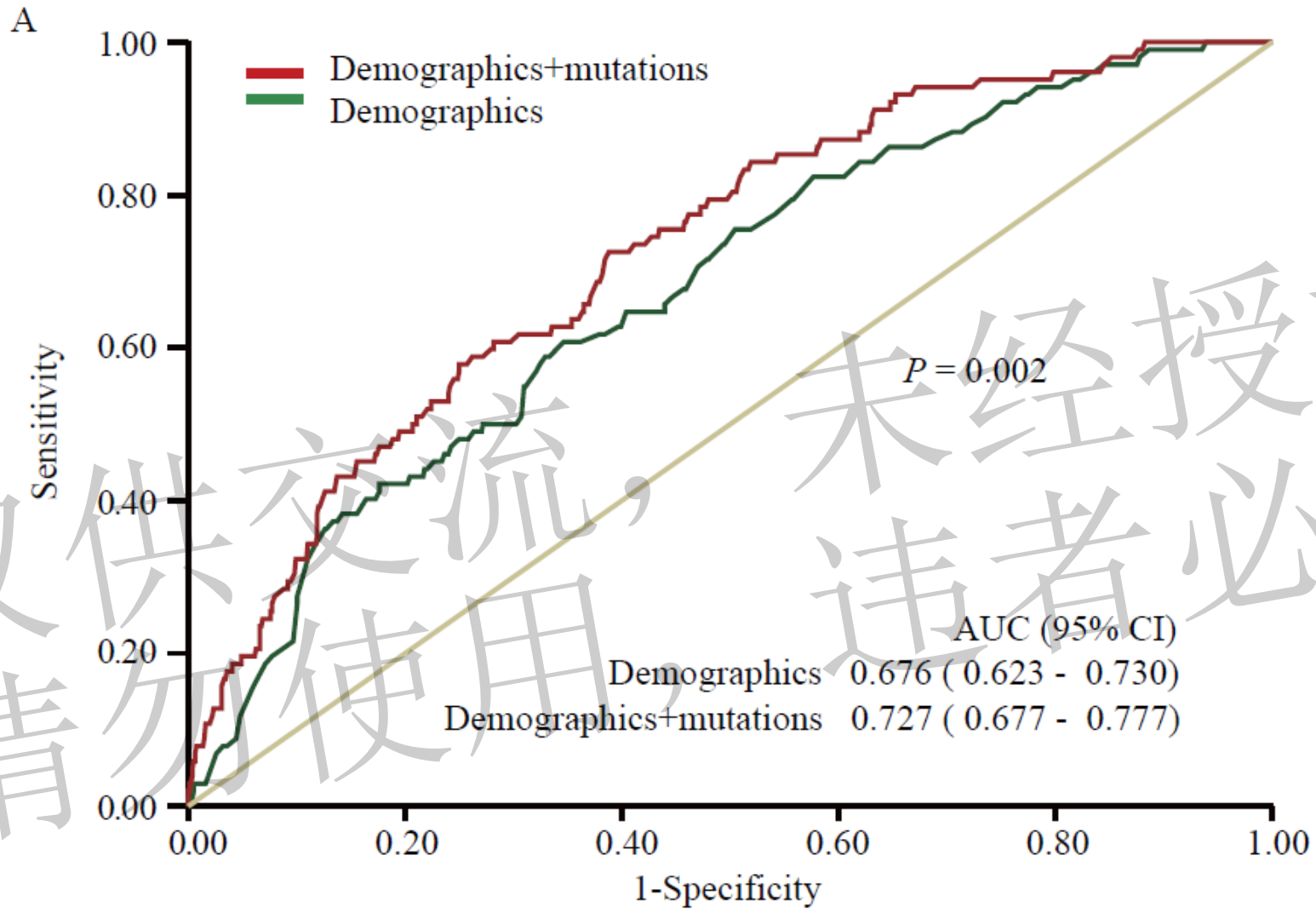


No. at risk

-/-/-	293	278	272	258	198	142	68	10
-/-/+	285	260	248	228	182	118	65	10
+/-/+ or -/+/+	191	168	159	136	113	80	37	3
+/+/+	62	51	49	44	32	18	8	2

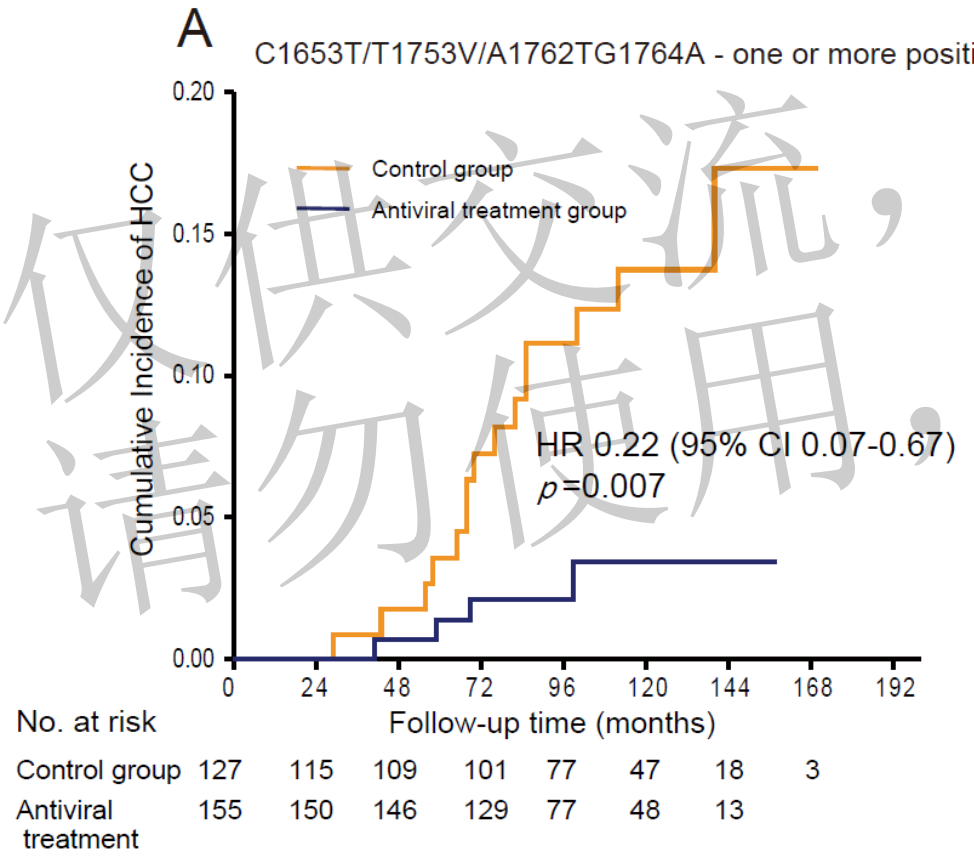


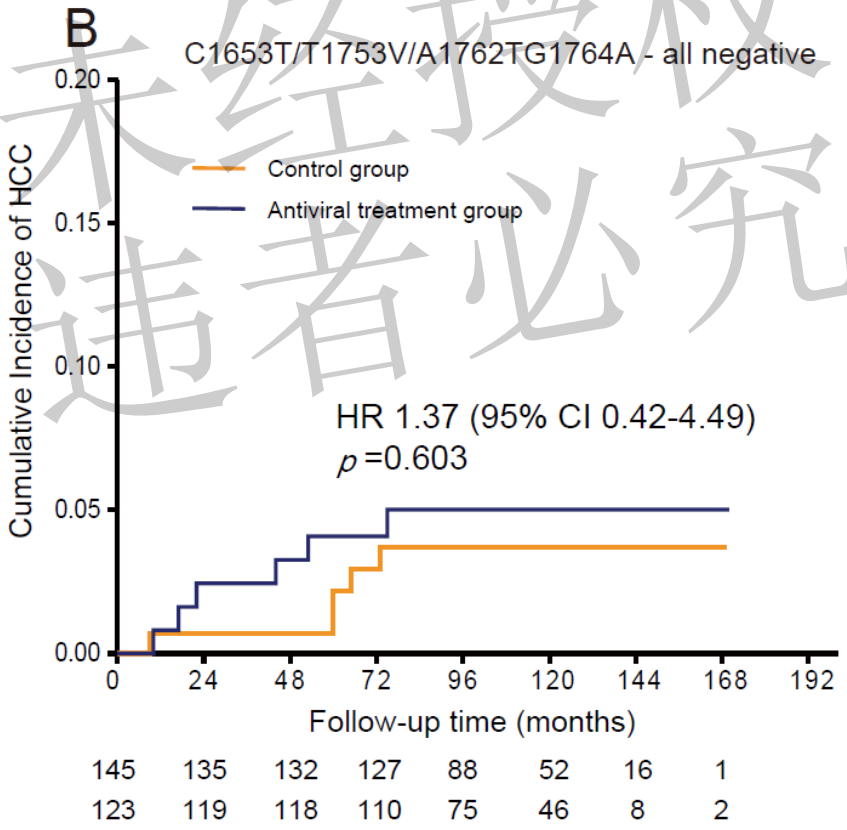
	293	278	272	261	200	142	68	10
	285	261	249	233	191	124	67	10
	191	175	165	144	123	83	38	3
	62	52	51	47	37	22	9	2

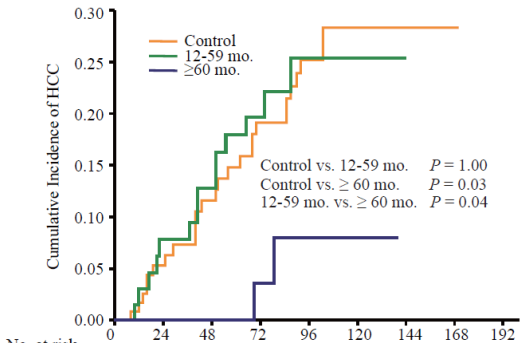


Baseline combo HBV mutations significantly increase the accuracy of HCC prediction by demographics

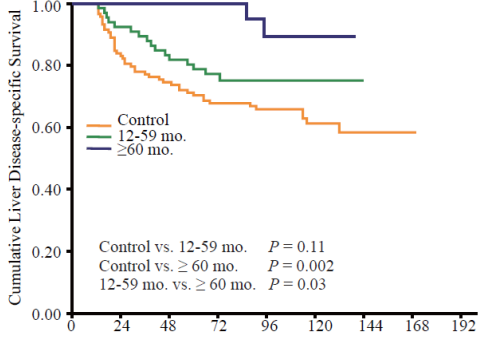
Standard antiviral treatment (≥48 wks) greatly reduced HCC occurrence in those with the combo HBV mutation, not in those without the mutations





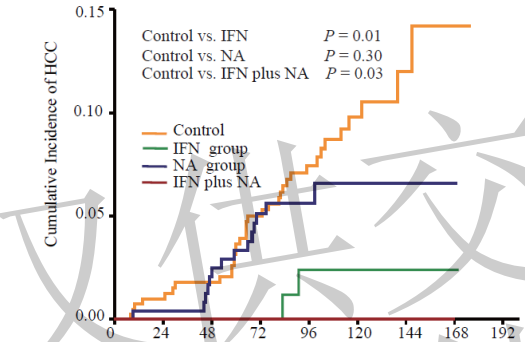


No. at risk								
Control	118	95	83	71	55	32	11	1
12-59 mo.	66	57	51	34	22	13	2	
≥60 mo.	32	32	32	24	14	8		



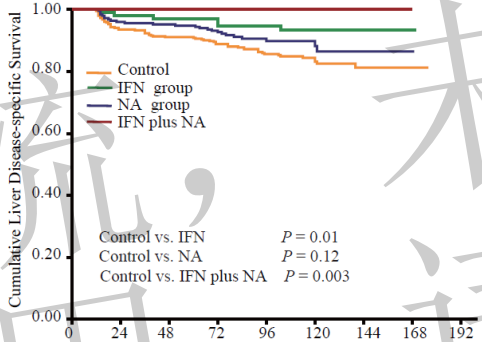
	118	99	88	76	63	36	12	1
	66	61	55	38	26	14	2	
	32	32	32	25	15	8		

C



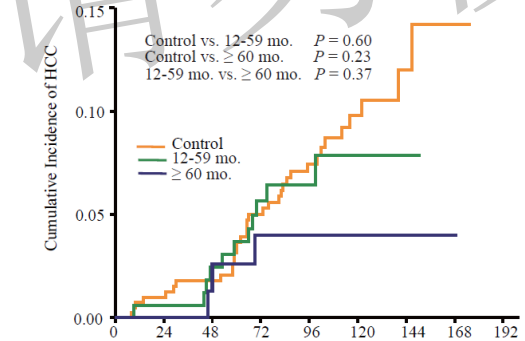
No. at risk								
Control	403	375	366	345	253	131	49	7
IFN group	97	95	94	91	78	58	17	4
NA group	249	239	233	199	111	54	17	1
IFN plus NA	57	57	57	48	34	14	3	1

D



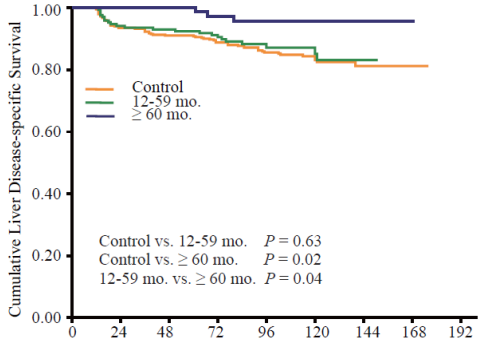
	403	377	367	351	260	137	52	8
	97	95	94	91	80	58	17	4
	249	239	237	206	114	55	17	1
	57	57	57	48	34	14	3	1

E



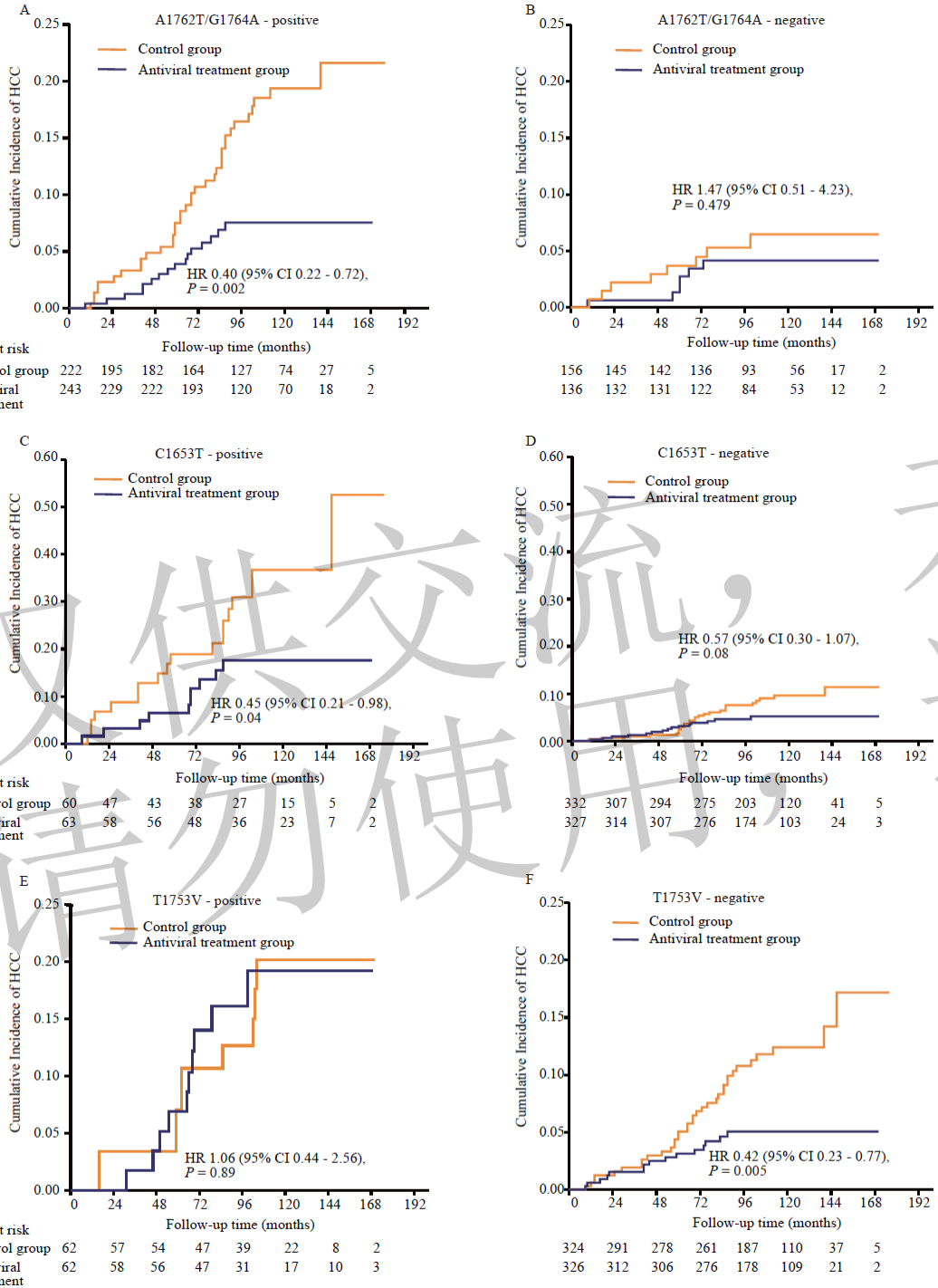
No. at risk								
Control	403	375	366	345	253	131	49	7
12-59 mon.	172	162	157	131	75	43	14	
≥60 mon.	77	77	76	68	36	11	3	1

F



	403	377	367	351	260	137	52	8
	172	162	160	136	77	44	14	
	77	77	77	70	37	11	3	1

Prophylactic effects of antiviral treatment with NA < or ≥48 wks or IFN on HCC occurrence and liver death

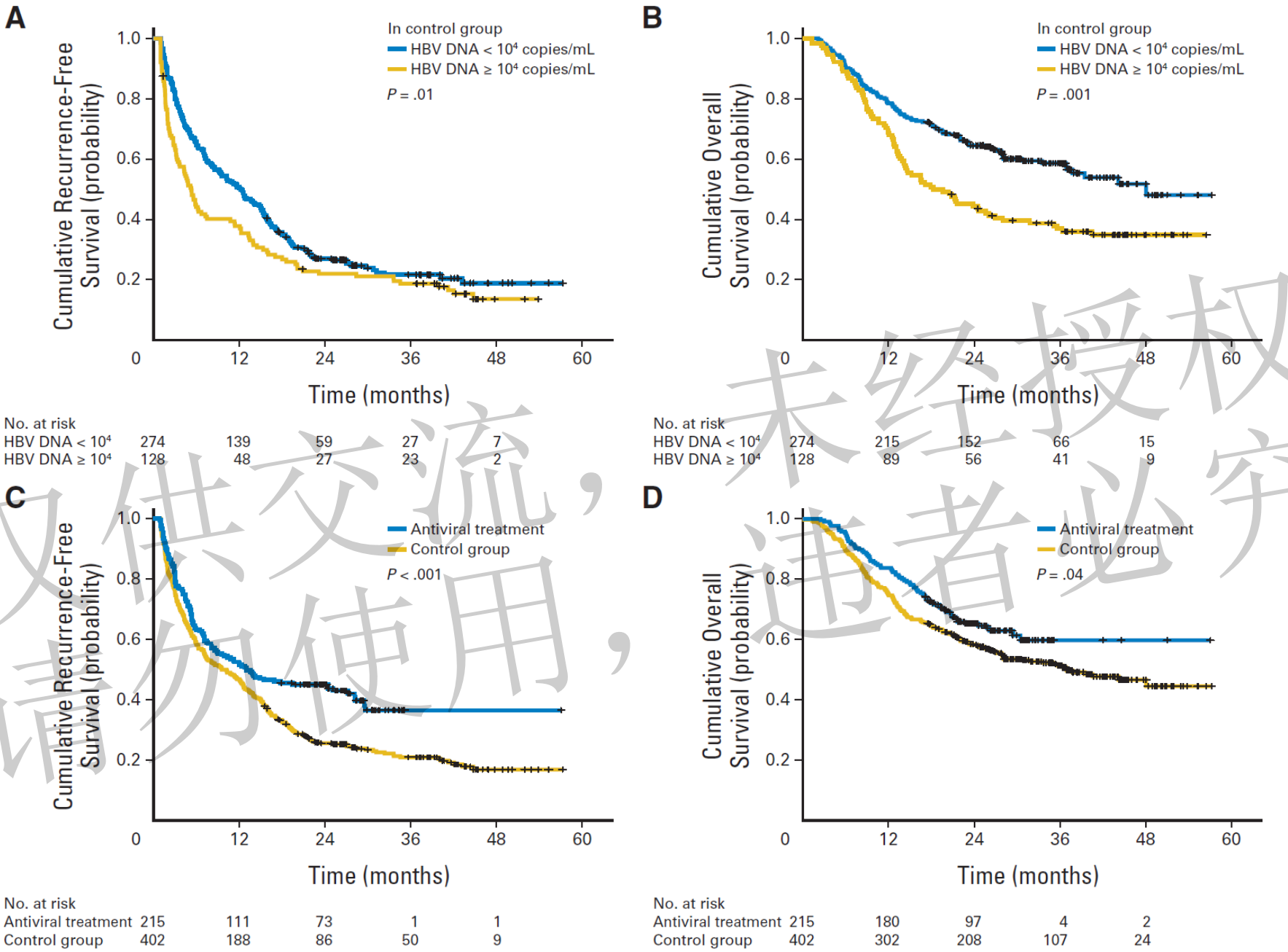


Prophylactic effects of standard antiviral treatments on HCC occurrence in HBG-infected patients with different viral mutation

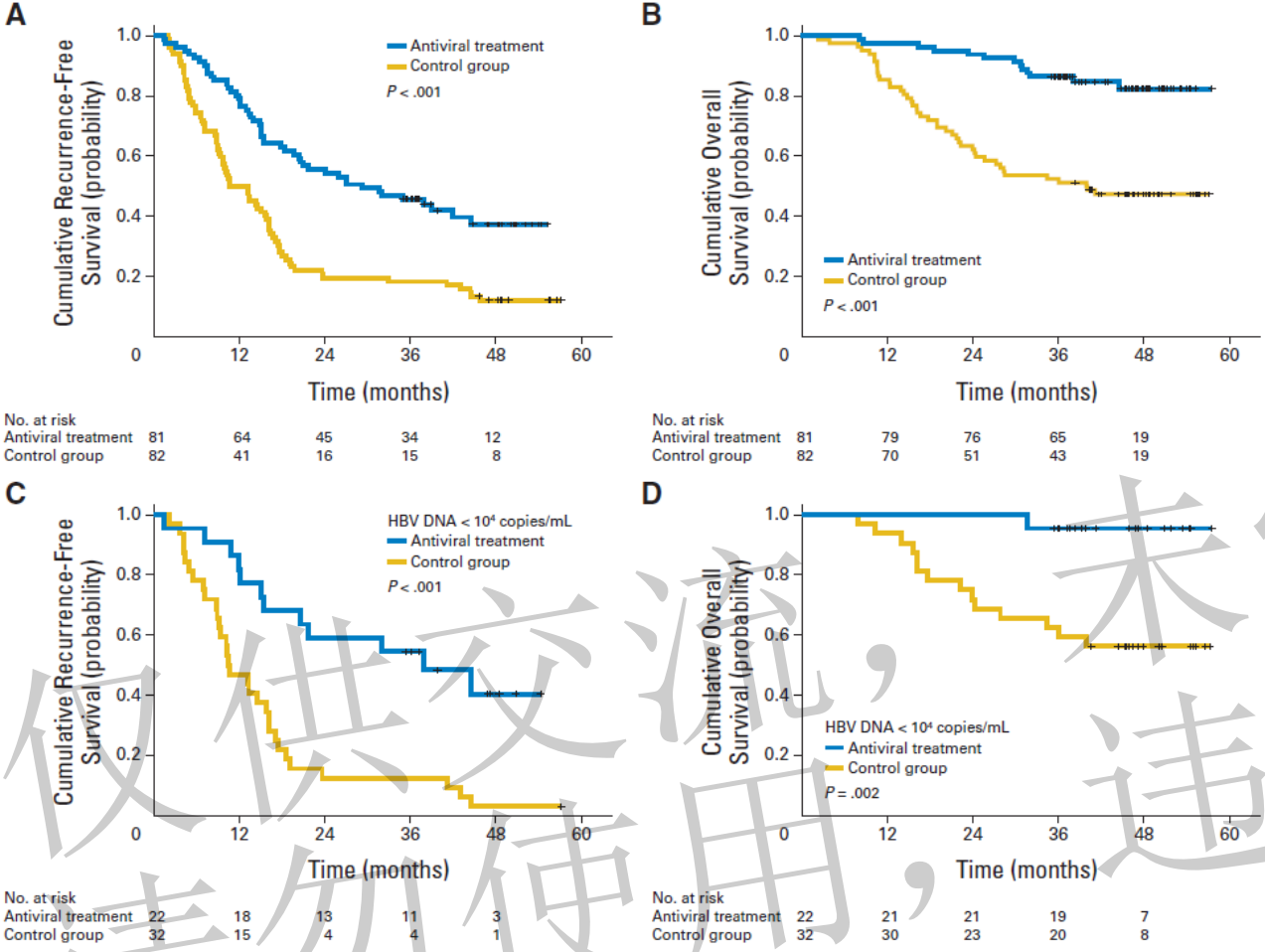
Cohort 2: Prophylactic effect of antiviral treatment of postoperative prognosis of HBV-HCC patients

- Cohort: 617 (antiviral, 215; control, 402) HBV-HCC subjects after curative surgery, followed up for 23.83 months;
- RCT: 163 (antiviral, 81; control, 82) HBV-HCC subjects after curative surgery, followed up for 39.93 months.

J Clin Oncol 2013;31:3647-3655



Cohort study: high viral load predict an unfavorable prognosis and N.A. treatment significantly improve the survivals



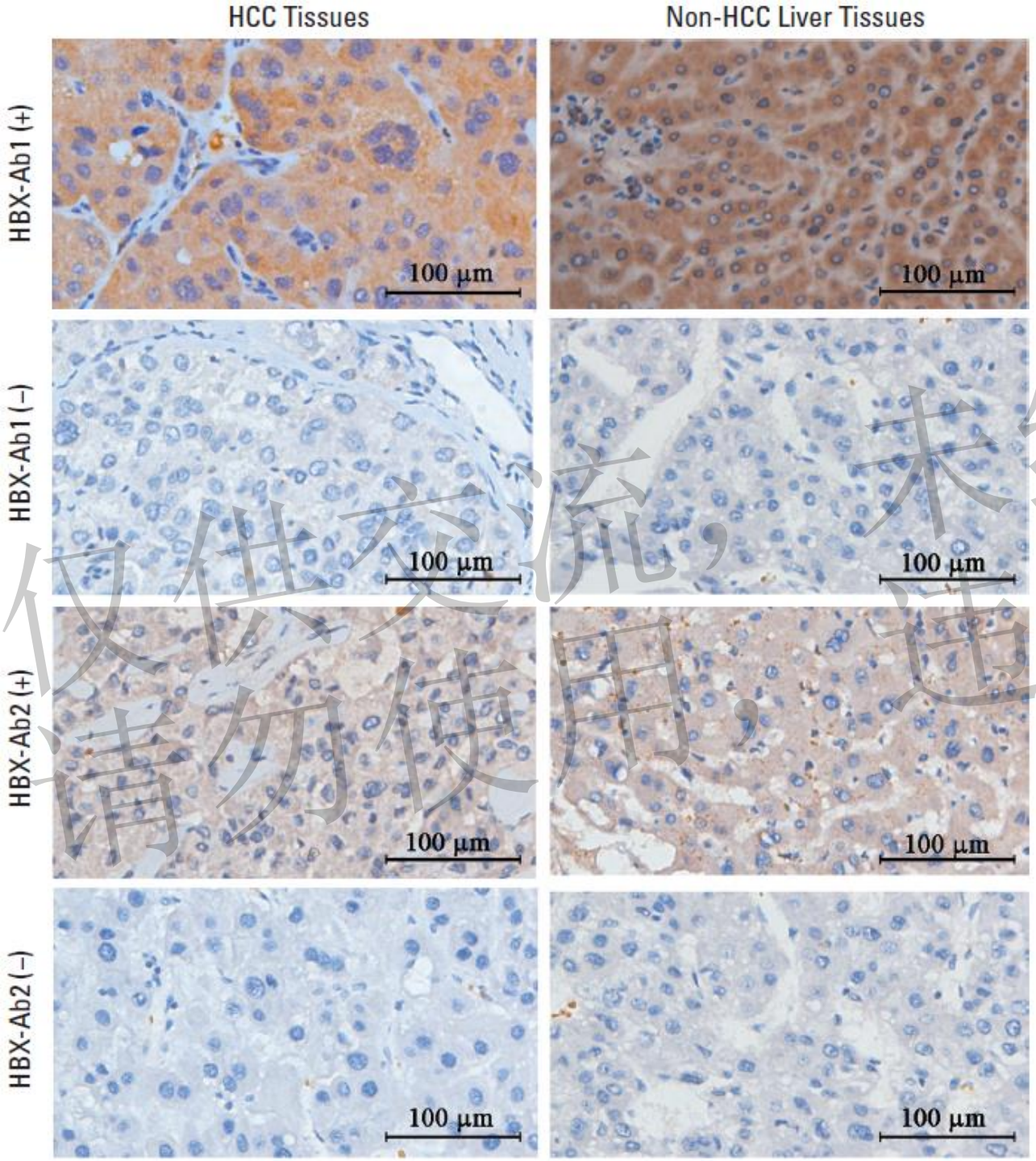
RCT: N.A. treatment significantly improve the survivals

Postoperative NA treatment significantly decreased inflammation, improve liver function

Table 4. Effect of Postoperative NA Treatment on the Recovery of Liver Function and HBV DNA Level in the RCT

Variable	Antiviral Treatment		Control		
	Median	IQR	Median	IQR	*P*
Bilirubin, μmol/L					
Preoperative	14.40	11.25-18.8	15.25	11.1-18.73	.74
1 month after surgery	17.90	13.45-23.90	21.25	16.3-32.18	.004
6 months after surgery	14.56	11.96-18.36	18.85	14.48-24.60	< .001
Direct bilirubin, μmol/L					
Preoperative	5.10	3.95-6.95	5.20	4.00-7.10	.98
1 month after surgery	8.60	6.30-12.35	10.15	7.15-14.83	.11
6 months after surgery	5.54	4.29-9.54	9.40	6.88-14.00	< .001
Albumin, g/L					
Preoperative	41.50	38.75-45.10	42.20	39.80-44.43	.47
1 month after surgery	33.90	30.85-38.35	33.6	31.43-36.38	.65
6 months after surgery	41.10	37.75-44.55	39.50	36.10-42.73	.02
ALT, U/L					
Preoperative	47.30	33.85-65.95	37.45	26.38-56.35	.02
1 month after surgery	58.05	47.62-69.51	67.25	56.61-79.56	.002
6 months after surgery	40.30	29.15-57.20	59.85	44.08-76.48	< .001
AST, U/L					
Preoperative	42.10	32.40-67.10	41.45	30.23-64.93	.48
1 month after surgery	41.54	29.26-57.12	54.14	33.39-65.32	.03
6 months after surgery	41.66	32.76-55.96	50.40	37.85-75.38	.004
HBV DNA, $\log_{10}$ copies/mL (mean ± SD)					
Preoperative	4.88 ± 1.27		4.58 ± 1.43		.16
1 month after surgery	4.31 ± 0.87		4.68 ± 1.47		.05
6 months after surgery	3.36 ± 0.68		4.66 ± 1.38		< .001

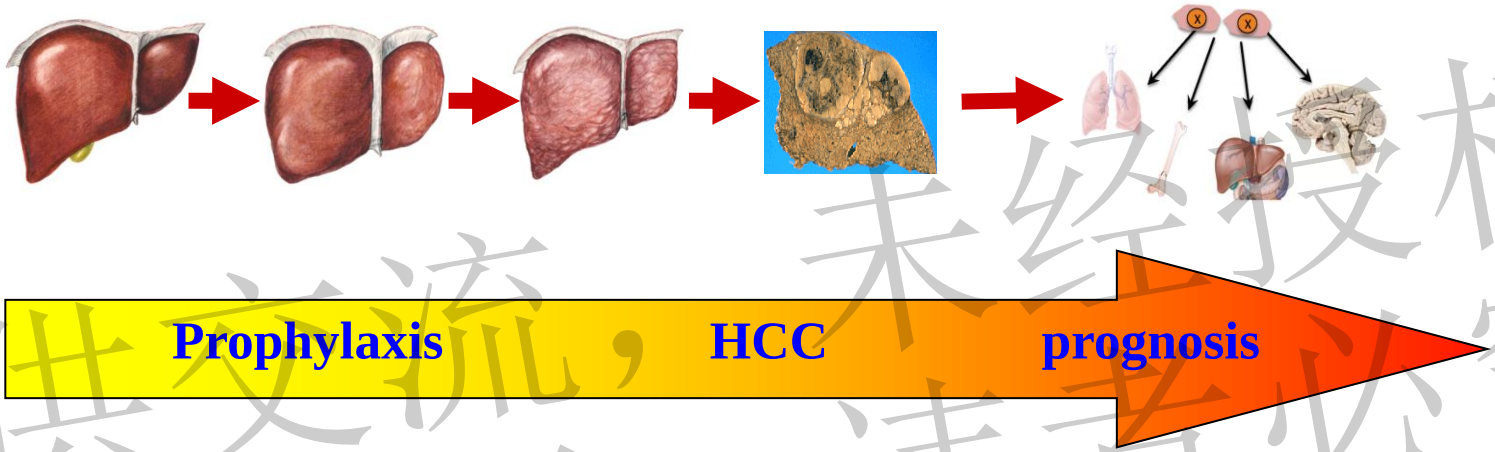
Abbreviations: HBV, hepatitis B virus; IQR, interquartile range; NA, nucleotide/nucleoside analog; RCT, randomized clinical trial; SD, standard deviation.



1. The expression of Ct-HBx was more frequent in HCCs than in adjacent hepatic tissues (P<0.001);
2. The expression of Ct-HBx in adjacent hepatic tissues was significantly associated with a reduced recurrence-free survival in the antiviral group (P<0.001).

J Clin Oncol 2013;31:3647-3655

Summary



- **For HBV-HCC, a fatal disease, prophylaxis is the hope of reducing HCC and death;**
- **"Cancer *Evo-Dev*" in HBV-induced HCC pave the way for prophylaxis, prediction, as well as targeted treatment;**
  - To identify what kind of HBV-infected subjects will develop HCC
  - To testify what kind of prophylactic treatment will reduce the risk of HCC
  - To specifically target key pathways that drive the evolution of HCC