being siRNAs, but still has its own limitations like silencing off targets and no access to nuclear genes. Hence among alternatives, catalytic nucleic acids are the best candidates to bet upon. The aim of this study is to use the catalytic nucleic acids like DNAzymes and RNAzymes as a candidate to have a control on the replication of influenza virus in the host cells.

**Methods:** The M1 gene of A/PR/8/34 (H1N1) strains were cloned in pCDNA 3. The computer based secondary structures of RNA were analyzed to design the DNAzymes with 10-23 catalytic motifs and the hammerhead Ribozymes. The DNAzymes and Ribozymes were used in combination.

**Results:** The DNAzymes were able to cleave the M1 RNA at 137 nt position whereas Ribozymes targeted at163 nt position in the same target. These catalytic nucleic acids were highly efficient under the simulated physiological conditions. When DNAzymes and Ribozymes were used in combination the cleavage was enhanced as compared to when they were used alone. Further an siRNA-Ribozyme construct was also designed. We have also demonstrated the modulation of the expression of target gene in controlled manner at RNA level by RT-PCR and FACs.

**Conclusion:** This combinatorial strategy can be used to design multi target DNA-enzymes and Ribozymes to delay the appearance of escape mutants because of the low probability of simultaneous mutations in both the target RNA sites.

**Free Paper Presentation 9 – Hepatitis B II**

**P-glycoprotein regulation in Hep3B cells by polypropenol could decrease the risk of hepatocarcinogenesis in HBV**

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**Background:** Over-expression of P-glycoprotein (Pgp) is associated with liver cancer development from HBV. The resent results are also in favour of the idea that glycoprotein synthesis in malignant tissues is limited by Dolichyl Phosphate (Dolp). The aim of the present study was to investigate the effect of polypropenol (PP) which provides a Dolp substitute in regulation of N-glycosylation on Pgp over-expression in the development of liver cancer in HBV infection.

**Methods:** Human hepatocytes, infected with HBV and human hepatocarcinoma HEP 3B cell line were used. Pgp was assessed by an immunohistochemical technique. Dolp fractions were analysed by HPLC methods.

**Results:** It is confirmed that plasmatic membranes of hepatocytes-cells contain 7.9-9.4% of Pgp (the total protein amount) as a resistance marker. HBV infected cells differ from normal hepatocytes in Pgp content by 4-5 times and Hep3B cells differ by 10-12 times. The study showed 5-fold Dolp decrease in HBV infected cells and 10-fold Dolp decrease in Hep3B cells. The investigations demonstrate that the situation can be changed by treatment with Dolp and PP. The Dolp concentration in HBV infected hepatocytes was returned to the normal level. It is established that Dolp in the concentration 10^5 M aids 6-8-fold reducing Pgp in membranes of HBV infected cells.

**Conclusion:** These results indicate that uncontrollable accumulation of Pgp in HBV infected cells can be overcome using simulation with Dolp substitution. Polyprenol usage can open up possibilities in liver cancer prevention in HBV infection.
Regulatory polymorphisms in the IL-10 gene promoter and HBV-related acute liver failure in the Chinese population

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Background: Recent reports indicated that high levels of IL-10 contribute to the monocytes paralyzation and poor clinical outcome in acute liver failure (ALF).

The aim of this study was to determine the possible association of the polymorphisms in the IL-10 gene promoter with the susceptibility to HBV-related ALF.

Methods: The IL-10 gene promoter polymorphisms were genotyped in 414 unrelated healthy blood donors, 367 asymptomatic HBV carriers and 345 HBV-related ALF patients using the polymerase chain reaction-restriction fragment length polymorphism assay or amplification refractory mutation system-polymerase chain reaction. Functional analyses were conducted to verify the biological significances of the associated genetic variations.

Results: The allele frequencies of IL-10 -592C and -819C were significantly higher in HBV-related ALF patients than in blood donors and asymptomatic HBV carriers. Logistic regression analysis and stratification analysis with adjustment for age and sex indicated that the polymorphisms of A-592C and T-819C were associated with susceptibility to HBV-related ALF (P = 6.9 x 10^-6, 1.5 x 10^-3, 1.5 x 10^-3). The mean number of significant polymorphisms was 10 in the Chinese population in chronic hepatitis B in Khotan area, Xinjiang, China, which may be of significance in the studies on population genetics and disease association.

OL-054 Identification, distribution, antimicrobial susceptibility and molecular epidemiology of new serotype 6C Streptococcus pneumoniae in China

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Objectives: To investigate the identification methods of the new serotype 6C; Surveillance the antimicrobial susceptibility and molecular epidemiology of the new serotype 6C Streptococcus Pneumoniae from China.

Methods: The new serotype 6C Streptococcus Pneumoniae were determined by multibean assay in USA, Serological method by multiplex PCR amplification of capsular gene targets. Antimicrobial susceptibility to erythromycin and beta-lactam antibiotic were detected by E-Test. PCR was used to detect genes ermB and mefA. The typing methods of molecular biology were used to show the homology of all serotype 6C strains.

Results: 10 serotype 6C isolates were determined from 100 serotype 6A by the three methods, 9 were isolated in Beijing and 1 in Guangzhou, 6 strains were isolated in Beijing in 1997. All strains of serotype 6C were resistant to erythromycin but susceptible to beta-lactam antibiotic. 8 isolates were detected only ermB and 2 only mefA. All isolates of serotype 6C were distributed 3 Pbps types, 4 PFGE types and 4 MLST types respectively. Pbps types I, PFGE types I and MLST types I include 6, 7 respectively. Pbps types I, PFGE types I and MLST types I include 6, 7 strains respectively.

Conclusions: There were three methods can be used to determine the new serotype 6C Streptococcus Pneumoniae in China. The new serotype 6C could be mainly identified ten years ago in China and all strains were susceptible to beta-lactam antibiotic but resistant to erythromycin. The gene typing present a diversity, but more isolates were in one type.