either the NS5B apoenzyme or P495 site inhibitor bound NS5B. This pattern of trypsin sensitivity and resistance correlates well with NS5B polymerase activity: activity is associated with conditions that stabilize the enzyme to trypsin treatment, and inhibition is associated with an open conformation that is specifically cleaved by trypsin. Co-crystal structures of NS5B and P495 site inhibitors have clearly shown displacement by inhibitor of the D1 finger loop from a thumb binding pocket. Our work demonstrates that inhibitor binding, which is 30 angstroms from the active site, induces a conformational change near the active site, a change also induced by template. The picture that emerges from the tryptic digest profile in conjunction with activity and binding data, defines the relationship between the binding of RNA template, RNA template-primer, NTP and P495 site inhibitors, and the enzymatic activity of the HCV NS5B polymerase. This relationship provides evidence for a detailed mechanism of inhibition by showing the status of the finger-loop in the "opened" or "closed" state in the presence of both ligands and inhibitor. The results suggest HCV NS5B possesses a hinge region similar to the hinge found in the DNA polymerase I family and that the conformation of the hinge directly impacts NS5B activity.

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Neuraminidase Inhibitor Susceptibility of Swine Influenza A Viruses Isolated Between 1981and 2008 in Germany

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The recently emerged pandemic influenza A virus (FLUAV) of subtype H1N1 provides evidence that swine FLUAV can donate genes for human pathogenic viruses, including those conferring drug susceptibility or resistance. The new H1N1 reassortant comprises the M2 as well as the neuraminidase (NA) gene of European swine viruses. As a result, it became amantadine resistant but is neuraminidase susceptible. This underlines the need of antiviral studies with swine FLUAV. In the present study, NA inhibitor (NAI) susceptibility of ~240 serologically typed swine FLUAV circulating in Germany between 1981 and 2008 was analyzed in chemiluminescence-based neuraminidase inhibition assays. The mean 50% inhibitory concentration of oseltamivir and zanamivir determined for these swine FLUAV strongly corresponds with that of human strains. Some isolates with lower drug susceptibility were identified which will further characterized genetically. Previously, an additional glycosylation site at Asn163 of H1 was shown to severely hamper the antiviral effect of NAI in MDCK cells. It was also detected in a certain number of German H1N2 isolates without resistance mutations in the NA gene. Plaque reduction assays and immunohistochemical detection of viral nucleoprotein were applied to compare the inhibitory effect of both drugs against three H1N1 isolates without and two H1N2 isolates with Asn163 in regard of virus titre and viral spread in MDCK cells. The results confirm a markedly reduced oseltamivir and zanamivir susceptibility of the H1N2 isolates in cell culture-based assays. Using the H1N1 isolate A/swine/Potsdam/15/81 and the H1N2 isolate A/swine/Bakum/1832/00 as examples, the inhibitory effect of oseltamivir was studied in 6–8-week-old female BALB/c mice and 11-week-old pigs to get an answer to the question whether these mutations would also affect negatively antiviral activity of NAI in vivo. The results indicate an antiviral effect of oseltamivir against both swine FLUAV in mice as well as the natural host.

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Liver Biopsy Tissue—Real Time Polymerase Chain Reaction (RT-PCR) Viral Load is the only *Gold Standard* Diagnostic *Assay* in Inactive Viral Hepatitis Patients

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Inactive carriers of Viral hepatitis B and C infections being followed up every 24 weeks with Serum Alanine Aminotranferease (ALT). Prolonged waiting without treatment; may progress to cirrhosis liver/hepato cellular carcinoma; because of progression of pathology and viraemic episodes. Same patients' sera containing HBsAg, anti-HBcIgM, HBeAg, anti-HBeAg, and anti-HBs in Hepatitis B carriers in various combinations; transient disappearance of anti-HCV and Sr. ALT levels fluctuate; causes indecisiveness in treatment choice. A *New Positive Approach* is; culling out hepatotropic viruses; HBV-DNA and HCV-RNA in their abode; Biopsy tissue and sera are assayed for viral load by RT-PCR; Rotor gene RG 3000 used with selected Primers. Histopathology for histological Activity Index and Stage of fibrosis assessed. To beat resistant mutant viruses and reduce duration of treatment two newly proved antiviral agents Chloroquine & Nitazoxanide are added with protocol drugs.

Subjects: 26 males and 6 females; age: 24–64 years. HBsAg carriers: 31 anti-HCV carrier: 1.

Results: Sr. RT PCR HBV-DNA: 12 <100 copies Sr. RT PCR HBV-DNA: 19 500–1324 copies; liver tissue HBV-DNA 31: 1732–112,39,82,825 copies Sr. and liver tissue RT-PCR HCV-RNA −1 negative.

Liver biopsy: Fatty liver: 14, mild inflammatory changes: 5, chronic active hepatitis: 11, no pathological changes: 2.

Sustained virological response (SVR): 2 patients had <100 copies—HBV-DNA in 17 and 28 weeks which are appreciably short duration treatment

Discussion: High serum level of HBV DNA more than 1000 copies denotes high activity of the disease. But occult sub clinical activity is elucidated by the liver biopsy tissue RT-PCR viral load of 1732–112,39,82,825 copies denotes in 31 patients there is continuous activity. This leads us to initiate a combination of antivirals for radical cure and prevent complications. The patient with anti-HCV positive in serum but RT PCR is negative in serum and liver tissue denotes a false positivity. Thus avoid antiviral therapy.

Conclusion: The above said facts dictate the new MOTTO "Liver Biopsy tissue RT-PCR assay is the only GOLD STANDARD ASSAY" for definitive diagnosis and SVR in inactive viral hepatitis.

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