Technical Abstract

Nucleonics, Inc.'s NUC B1000 an eiRNA-based product candidate is designed to treat patients chronically infected with Hepatitis B. Despite the availability of antivirals and immunomodulator,s there still is a significant unmet need for the treatment of chronic Hepatitis B infection. eiRNA-based drugs have the potential to provide therapies that are based on alternate mechanisms of action than currently used drugs.

The drug product will consist of the NUC 500-001 plasmid DNA, the active drug substance formulated with the delivery excipient NUC 600-001. The NUC 500-001 plasmid DNA is designed to silence HBV gene expression thereby inhibiting expression of viral antigens and inhibiting viral replication. The excipient is designed to permit more efficient delivery of NUC 500-001 to infected hepatocytes. Nucleonics is proposing to initiate human trials in chronically infected HBV patients. The proposed first in human clinical trial will consist of a safety and tolerability study of NUC B1000 in chronically infected HBV patients. The Phase 1 study will be a dose escalation study with a subset of cohorts receiving a repeat dose

HBV and RNAi Therapy:

HBV Background: Approximately 5% of the world population is infected by the hepatitis B virus (HBV), a DNA virus belonging to the Hepadnavirus family. HBV is transmitted through sexual contact, illicit intravenous drug use, and by perinatal exposure of the newborn to maternal blood. Although most adults infected by HBV recover spontaneously, 5 to 10% of infected adults develop persistent infections. In contrast, maternally transmitted HBV results in chronic infection in 90% of exposed infants. Consequently, more than 300 million people worldwide are chronically infected by HBV, and there are approximately 1 million chronically infected patients in the United States. About 25% of these patients will die as a consequence of cirrhosis and/or hepatocellular carcinoma (HCC).

Although a prophylactic vaccine is currently available, there are few treatment options for chronically infected individuals. In addition, these treatment options have drawbacks and are not efficacious in the majority of individuals. IFNα 2b is one of the few approved treatments for chronic HBV but is only recommended for a set of patients within a narrow clinical profile. IFNα has antiviral activity and the ability to stimulate the immune system to eliminate a subset of infected hepatocytes. It has potentially dose-limiting side effects. Oral nucleoside analogues such as lamivudine, entacavir, adefovir and others have shown some promise for the treatment of chronic hepatitis B. However, while these drugs significantly lower viral titers, they do little in the way of decreasing HBV antigen load, the primary cause of liver inflammation. In a comparison of the antiviral agents, there appears to be no correlation between better reduction of viral titer and seroconversion rates. In addition, oral antiviral therapy is limited by its ability to select viral escape mutants; lamivudine resistant mutants occur in nearly 80% of treated patients, while drug resistance appears at a lower frequency with other antivirals.

In summary, there is a significant unmet need in the treatment of chronic HBV infection. Current treatment options suffer from low efficacy, side effects, and the selection of drug resistant mutants. Moreover, these treatments do not address the underlying disease of antigen mediated immune attack on infected hepatocytes.

RNAi background: RNA interference (RNAi) represents an innate and universal cellular phenomenon triggered by dsRNA. Nucleonics' approach is to express short hairpin RNAs

(shRNAs) from a non-replicating plasmid DNA. Transcription of the encoded shRNAs is directed by endogenous RNA polymerase III. These shRNAs are processed in the cell into siRNAs that bind RISC, thereby activating it. The mechanism although not completely understood suggests that only one of the strands of the initially bound siRNA is retained in RISC. Activated RISC binds mRNA molecules that are complementary to the siRNA sequences within activated RISC, and catalyzes the cleavage of bound mRNA. Cleavage of the mRNA renders it non-functional in its participation with the protein translation machinery. Following cleavage of the mRNA, activated RISC dissociates to bind yet another mRNA molecule to effect multiple cycles of the binding and cleavage process.

Nucleonics and several other laboratories have demonstrated exquisite sequence complementarity requirements between the contained siRNA sequence and the targeted mRNA sequence to induce the RNAase mediated hydrolysis of the targeted mRNA.

Rationale for RNAi as a therapeutic: Liver pathology associated with HBV infection is primarily immune-mediated, and is thought to be due to cell-mediated immune responses directed against infected hepatocytes. During chronic infection, HBV DNA is frequently found to be integrated into host DNA. Both integrated and non-integrated HBV DNA (cccDNA) are transcribed by human RNA Polymerase II. Translation of cccDNA derived transcripts results in the expression of all viral antigens while translation of integrant derived transcripts results only in expression of X protein and HBsAg. These proteins contribute significantly to antigen load within infected cells. Current antiviral treatments with nucleoside analogues such as lamivudine suppress viral titers without affecting antigen load. Indeed, as would be expected even after reduction of viral titer by these drugs, antigens continue to be expressed from integrants and persisting cccDNA allowing hepatocytes to remain susceptible to immune attack.

The chronic inflammation induced by the immune response to persistent antigen expression is thought to contribute importantly to the development of cirrhosis, fibrosis and HCC (hepatocellular carcinoma). Therefore, treatments that target antigen expression as well as viral replication offer a significant advantage over existing treatment options because they would decrease the visibility of hepatocytes to the immune system thereby decreasing the severity of chronic inflammation/hepatitis. IFNα treatment results in decreased antigen production through inhibition of protein synthesis nonspecifically and is therefore not so well tolerated. RNAi-based therapies are specific and therefore predicted to be better tolerated and have the potential to be beneficial for healthy carriers as well as a subset of patients who are at risk of developing chronic active hepatitis but for whom no current therapy exists. In addition, a subset of patients exhibiting non-immune mediated liver pathology caused by L-HBsAg over-expression is also likely to benefit from an RNAi-based treatment.

Therapies employing RNAi have the potential to inhibit viral replication and down-regulate antigen expression simultaneously and by mechanisms that are different from those of currently available therapies. Unlike small molecule therapeutics which target a specific viral activity and hence a specific portion of the virus, plasmid NUC 500-001 was designed to target four separate regions of the viral genome.

Drug Substance NUC 500-001

Following introduction of the NUC 500-001 into the cell, short shRNAs are transcribed, yielding multiple virus-specific siRNAs. These siRNAs bind and activate the RNA-induced silencing complex (RISC) to specifically target and cleave the corresponding viral RNAs within the infected cell. The RNAse(s) associated with activated RISC degrade the viral messenger RNA, thereby inhibiting viral replication and gene expression. Following intravenous administration of

the formulated product, NUC B1000 injectable suspension, the drug substance NUC 500-001 is internalized into hepatocytes and transcribed by the host RNA polymerase III to yield multiple siRNAs that target four separate target sequences in multiple HBV mRNAs.

Clinical Plans: NUC B1000 Injectable Suspension is being developed as a treatment for chronic Hepatitis B infection. The approach utilizes targeting of multiple conserved sequence epitopes (sequitopes) in the viral RNAs such that it is expected to be broadly effective against wild type and emergent mutant strains of the HBV.

The Phase 1 study will focus on the evaluation of basic safety in patients with mild to moderate active disease over a dose range of two orders of magnitude to assess the potential, if any, for acute toxicity and an immunologically induced serious adverse response. Within this context, the Sponsor believes that dosing of humans with minimally active chronic Hepatitis B using a single intravenous dose can evaluate the potential acute toxicity of NUC B1000 and also begin to addresses the safety parameters associated with drug efficacy.

Completed preclinical toxicology studies to date in mice indicate no acute or long term adverse effects following single and multiple dose administration with the exception of perivascular inflammation at the injection site with repeated administration.