DEVELOPMENT AND COMMERCIALIZATION OF HEPATOCYTE TARGETED
DRUG DELIVERY VEHICLE FOR PHARMACEUTICAL APPLICATION

by

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Development And Commercialization Of Hepatocyte Targeted Drug Delivery
Vehicle For Pharmaceutical Application

Abstract

by

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Over 3% of the world's population is infected with the hepatitis C virus (HCV) and is a leading cause of death in developing nations. We have been unable to develop a vaccine against HCV infections unlike HAV and HBV since very little of the life cycle of the virus is known and there have been no culture systems available for HCV until recently. The current Standard of Treatment is not always effective and has significant side effects. The efficacy is low due to the limitations on administered interferon, which causes the side effects. These physical as well as psychotropic side effects lead to low patient compliance and lower efficacy of viral knockdown. Here we try and develop a solution that attempts to deliver interferon to the liver and localize the drug by molecular targeting. This could significantly increase efficacy and reduce side effects of the current standard of cure.
2. Introduction

Of all the viral diseases effecting humans, Hepatitis is one of the leading causes of death in the developed nations. About 3% of the world's population is infected with the hepatitis C virus (HCV) and is at risk of developing liver cirrhosis and/or liver cancer. The transmission occurs through transmission of body fluids or by sharing of needles. The infection remains asymptomatic in the acute and early stages, which makes diagnosis difficult. After decades of infection and the unaware patient the frequency of transmission becomes much higher. After the disease progresses a significantly the patient faces symptoms which are due to severe damage done to the liver. This leads to cirrhosis or even liver cancer in the later stages of the disease. Very little of the life cycle of the Hepatitis C virus (HCV) is known and there have been no culture systems available for HCV until recently. This has impaired the development of therapies to treat HCV and vaccines and prevent infection. This treatment although not always effective, has significant effect on patient’s quality of life due to significant physical as well as psychotropic side effects. The low efficacy of the drug is due to the limitations of dosage that can be administered due to the significant side effects. Here we try and develop a formulation that uses liposomes and a Hepatocyte targeting molecule to deliver interferon to the liver and localize the drug. This could significantly increase available interferon to the liver, thus increasing efficacy and reduce systemically available interferon, thus reducing the side effects.
a. Biology of Viral Hepatitis

i. Viral Hepatitis:

‘Hepatitis’ etymologically is a Greek word, which implies injury to the liver characterized by the presence of inflammatory cells in the organ. The manifestations of such a liver disease include jaundice, which was characterized by Hippocrates in ancient Greece and was found to be infectious as early as the 8th century. Today there are many different forms of hepatitis known, which can viral hepatitis, autoimmune hepatitis, fatty liver hepatitis, alcoholic hepatitis and toxin induced hepatitis. Of the known different forms of hepatitis, viral hepatitis is the most common form that can cause severe liver damage and even death.

Records confirm that Hepatitis was found to be transmittable through blood transfusions as early as 1885\(^1\). In 1901-1902 the Reed Commission identified yellow fever due to a filterable agent (later identified as a virus), for hepatitis. After a series of outbreaks in the World War II, MacCallum\(^{11}\) in 1947 identified viral hepatitis into two types as Infectious and Serum hepatitis. In 1963 serum hepatitis and its cause were identified and named the virus Hepatitis B Virus (HBV). Today there are various viruses identified that cause hepatitis. These may or may not be related to each other in genetic makeup. Some of the known viruses causing hepatitis are RNA viruses while the other have DNA as their genetic material.
Some commonly known Hepatitis viruses can be listed as:

**Hepatitis A Virus**

Hepatitis A Virus belongs to the family of *Picornaviridae*. Like most Picorna viruses it has a single stranded RNA packed in a protein coat. There are no specific treatments for Hepatitis A but the patients are advised rest, avoid alcohol and fatty foods and remain hydrated. In the year 1991 the US, Centers for Disease Control and Prevention (CDC) reported an mortality rate for hepatitis A as low as 4 per 1000 cases for the general population which increased in patients infected over 50 years of age upto 17.5 cases per 1000. In 2007 there have been no diagnosed cases of chronic Hepatitis A and there are no cases of associated deaths. There are popular and well accepted vaccines available in the market for Hepatitis A virus. These are inactivated particles of hepatitis A virus, which help develop the immunity against the virus in the body. The vaccine is often administered where the 1st vaccination provides protection after two to four weeks after administration and the second administered after 6 months to an year after the 1st provides immunity against Hepatitis A for upto twenty years. The virus is transmitted through fecal-oral route via contaminated food and water and can be prevented by vaccination, good hygiene and sanitation.

**Hepatitis B Virus**

Hep B Virus belongs to the family of Hepadnaviridae. This is a family of Viruses that cause liver infections in humans and animals. The virus consists of an outer lipid envelope and a nucleocapsid of protein. Like most Hepadna viruses it has a very small genome which is a circular double stranded DNA. The genome is unusual because the
DNA is not fully double stranded. One end of the longer strand of the DNA is linked to the DNA Polymerase that has reverse transcriptase activity\textsuperscript{vii} which makes it one of the few known non retro viruses that use reverse transcription. Acute Hepatitis B infection usually does not require treatment because adults clear infection spontaneously but chronically infected individuals are candidates for antiviral therapy. Chronic infection with HBV causes severe damage to the liver and can lead to cirrhosis and even cancer. The drugs available in the market today for the treatment of Hepatitis B infection may not be able to clear the infection from the patient’s body but stop the virus from replicating and minimize irreversible liver damages like cirrhosis and hepatic cancer. In 2007 there have been 1.4million diagnosed cases of chronic Hepatitis B and contributed to a total of 3000 associated deaths\textsuperscript{viii}. Several vaccines have been developed for the prevention of Hepatitis B infection. The vaccines contain one of the viral envelope proteins, commonly known as Hepatitis B Surface Antigen (HBsAg). There are administered in 3 doses and may be re-administered if the patient does not respond to Anti-HBsAg level of 100mIU/ml or higher. The vaccination provides protection for upto seven years and appreciated immunological memory shown with adequate response to initial vaccination to protect for upto 25years\textsuperscript{ix}.Hepatitis B virus is a blood born pathogen that is to say it is transmitted through activities that involve percutaneous (i.e., puncture through the skin) or mucosal contact with infectious blood or body fluids.

**Hepatitis C Virus**

Hepatitis C Virus (HCV) belongs to the family *Flaviviridae*. This is a family of Viruses that is known to spread by arthropods but HCV belongs to a Genus *Hepacivirus*, which is the single virus in the genus. HCV like Flaviviruses in general has a single
stranded RNA as genetic material. This genome core is surrounded by a protein shell which is further encapsulated in an envelope of lipids. The genome of HCV has a single open reading frame (ORF) which along with the ribosomal entry site at 5’ region helps in coding a 3000 amino acid protein which is later processed by proteases into 10 functional smaller proteins. Due to a high rate of replication, and a lack of proof reading mechanism by the HCV RNA protease HVC has a high probability to mutate which may help in avoiding the host immune response and is also responsible for the diverse genotypes that are available. The infection remains asymptomatic in acute stages and symptoms if any resemble acute hepatitis, which are mild and non-specific, hence rarely lead to the diagnosis of Hepatitis C. About 15-25% of infected individuals clear the virus during the acute phase spontaneously\(^\text{x}\). The remaining individuals transform into the chronic phase, which is considered within 6 months of infection. Recent NIH consensus on Management of Hepatitis C (2002) concluded that 20% of chronically infected Hepatitis C patients develop cirrhosis within 20 years of infection. The virus is transmitted by blood-to-blood contact, thus unscreened blood transfusion remains a major cause of HCV infection in developing countries. Other major reasons include unprotected sexual contact and sharing of needles.

Current treatment of Hepatitis C available is the combinational therapy of Pegylted Interferon alpha along with the antiviral drug Ribavirin. The duration of the treatment varies from 28 to 48 weeks depending on the genotype of the HCV infection. However the rates of sustained virological response (SVR) in patients vary upon demographic and geographic distribution. Some aboriginal races have shown higher spontaneous clearance and higher response to therapy than others\(^\text{x}\). HCV has many
strains known which vary in distribution geographically. Main genotypic variations of the Hepatitis C virus can be classified into two broader categories of Main (1-6) and secondarly genotypes (7-11) as suggested by Simmonds et al. These have further quasispecies, which are discovered with time, for example Genotypes 7–9 have been isolated only from Vietnamese patients while genotypes 10 and 11 are found in Indonesia where as genotype 4 and 5 are almost exclusive to Africa. The genotype of infection can depend on modes of infection, demographics and geographic location for instance, Genotype 1A is more commonly found in North America where as 1B and type 2 are prevelant in Europe particularly in old age groups. The individuals infected through drug use are more likely to get infected with genotype 3A and 1A. The virus has more than 100 quasispecies found in the sub-Saharan and Southeastern Asia. Each of the six main genotypes of HCV is equally divergent from the others, differing at 31 to 34% of nucleotide positions on pair wise comparison of complete genomic sequences, and leading to approximately 30% amino acid sequence divergence between the encoded polyproteins and this difference is considered to be responsible for the difference in response to the standard of cure which remains the combinational therapy of Pegylated interferon along with ribavirin. Genotype 1 and 4 which are closely related seem to be

![Phylogenetic tree of the HCV with Main and secondary genotypes.](image)
less responsive to the interferon therapy than the other (2,3,5 and 6). Hence the duration of therapy for genotype 1 and 4 is higher (48 weeks) as compared to the other (24 weeks). There are no vaccines developed yet for preventing infection from the HCV for 3 primary reasons:

1. HCV has different genotypes and quasispecies that behave differently and do not respond to one vaccine antibody.
2. As discussed earlier, due to the lack of proof reading in the HCV RNA Polymerase, the virus mutates rapidly.
3. There has been no animal model or cell culture system available until recently when Martina Buck, Ph.D at University of California, San Diego announced a new culture system in July 2008.

**Hepatitis D Virus**

Hepatitis D is caused by a Delta virus known has Hepatitis Delta virus. The virus has a genetic material of a small single stranded, closed circular RNA and is considered as one of the smallest virus to infect mammals. It functions as a sub-viral satellite and infects as co-infection with Hepatitis B. The virus genome is about 1700 nucleotides enclosed in a Hepatitis B surface antigen as its capsid. It does not encode or capsulate its own polymerase and require a host polymerase that can utilize its RNA. Hepatitis D is very rare in most developed countries and if any, is associated with drug abuse. The Delta virus infections are more common in mediterranean countries, sub Saharan Africa and some parts of South America.

Hep D Virus is transmitted as a co-infection of Hepatitis B or a super-infection into a person previously infected with HBV. The modes of infection thus are identical to
HBV infection by exchange of infected bodily fluids and exposure to contaminated sharps. It has a high mortality rate since it is a co-infection or a super-infection of HBV. Super-infection causes Fulminant viral hepatitis in acute conditions and is the most severe form of the acute disease. It is about 10 times more common with HDV/HBV infection than any other viral hepatitis. Chronic infections often lead to carcinomas and cirrhosis. No specific treatment is available for the disease. Larger doses of interferon causes remission but the patients often remain positive for HDV RNA. The effect is considered as a response of HBV to the interferon. Since the delta virus is dependent on Hepatitis B infection for polymerase and encapsulating surface antigen, vaccines that prevent infection from Hepatitis B are considered to protect against delta virus infections in highly affected regions of the world.

**Hepatitis E Virus**

Discovered first in 1955 in South Asia, the virus belongs to a viral genus of *Hepevirus* and has not been assigned a family in classification. The virus is has a non-enveloped genome of single stranded RNA with an isometric capsid.

Like Hepatitis A virus, HEV has a fecal oral route. The infection however is self limiting and does not cause chronic infection. Has an incubation period of 3-8 weeks. Infection and mortality increases upto 20% in pregnant women in the 3rd trimester. Typical symptoms of Hepatitis E infection include jaundice, hepatomegaly and other gastro-intestinal disturbances.
ii. Interferon

**Interferons (IFNs)** are natural proteins produced in the body of most vertebrates by the immune system in response to foreign agents like parasites or foreign proteins. Interferons belong to the class of signaling molecules in the body like interleukins, neurotransmitters etc called Cytokines. Interferons are generally produced in the body, by cells in response to abnormally large amounts of double stranded RNA, which is generally a sign of infection. The presence of dsRNA triggers the innate immune system which is not present in detectable amounts in uninfected cells. This results in expression of interferon producing genes and subsequently interferon is produced and released to the neighboring cells. It triggers the genes that code for the interferon. Interferon is thus made and secreted to the various surrounding cells. This form of signaling to the receptor cells acts as a ‘warning’ about viral infection. This ‘warned’ cell when infected by the virus slows or shuts down its protein synthesis, inhibiting viral replication.

There are two major classes of interferons according to the type of receptors that they signal to as per the Medical Subject Headings (MeSH). They are:

**IFN Type I**: These are receptor specific and bind to cell surface complexes. Homologous molecules to IFN type I are found in many organisms other than mammals. The mammalian types are designated IFN-α (alpha), IFN-β (beta), IFN-κ (kappa), IFN-δ (delta), IFN-ε (epsilon), IFN-τ (tau), IFN-ω (omega), and IFN-ζ (zeta). The two interferons used in Pharmaceutical applications are IFN-α (alpha) which is commonly known as Leucocyte interferon and IFN-β (beta) which is considered as a fibroblast interferon.
**IFN Type II**: This class of interferons has only one member as IFN-γ (gamma) which is a dimer of antiparallel strands. In contrast to interferon-α and interferon-β which can be expressed by all cells, IFN-γ is secreted by Th1 cells, Tc cells, dendritic cells and NK cells. With its association to immune system cells and for its anti-viral, anti-tumor and immune-regulatory properties it is also known as Immune Interferon\(^{xxv}\).

Collectively these interferons perform a variety of functions in the body like assisting the immune system, inhibiting viral replication, increasing antigen presentation to lymphocytes and activating other defense mechanisms in the body. The commonly accepted Interferon signaling pathway is JAK-STAT Pathway.

**iii. Ribavirin**

Ribavirin is an Anti-viral drug initially engineered for respiratory syncytial virus which is a viral infection of the lower respiratory track in humans. In combination therapy with Interferons it acts as a prodrug and when metabolized resembles purine nucleotides. It was inspired, in part by the discovery of purine like compounds that posses RNA interference activity. Ribavirin, however is not a naturally occurring compounds like other purine-like antibiotic molecules like coformycin and pyrazomycin. However, these natural molecules were never approved as drugs for use in humans due to high toxicity, but led to the development of other pharmaceutical products like Ribavirin. It is to be noted that the exact mechanism of Ribavirin function is not known however many suggestions have been given as to its effect on RNA and DNA viruses\(^{xxvi}\). Ribavirin is also known to enhance T-Cell mediated immunity against viral infections.
Ribavirin has some side effects like most drugs recommended for use in therapy against HCV. With its proposed mechanism of it is incorporated into the DNA and has a dose dependent effect on inhibiting DNA synthesis and other gene expressions.

iv. JAK-STAT Pathway

The JAK/STAT pathway is ubiquitous amongst vertebrates and participates in regulation of cellular responses to chemical stimuli like cytokines and growth factors, where JAK stands for Janus Kinases (named after the two faced Roman God of Doorways, Janus) and STAT stands for Signal Transducers and Activators of Transcription. The JAK-STAT pathway acts as a door and transduces the signal carried by the extracellular peptides to the cell nucleus to effect gene function. JAK activation can stimulate many cell function like proliferation, differentiation, cell migration and apoptosis. Many different chemical signals can stimulate the JAK-STAT pathway which occurs when the binding ligand induces the multimerization of receptor subunits into homo-multimers (as by some growth factors) or into hetero-multimers (as by cytokines and interferons). In the therapy of Hepatitis C the interferons currently used are Type I interferons, such as IFN-α (alpha), IFN-β (beta) that signal to the signal transduction and activator of transcription (STAT) through a
Janus kinase (Jak). This pathway is used to stimulate gene expression. Activation occurs when ligand binding induces the multimerization of receptor subunits for instance, JAKs phosphorylate STAT1 and STAT2, which are latent transcription factors that reside in the cytoplasm. Phosphorylated STATs enter the nucleus by a mechanism that is dependent on the Ran nuclear import pathway. Once in the nucleus, dimerized STATs bind specific regulatory sequences to activate or repress transcription of target genes. Thus the JAK/STAT results in a cascade of biochemical events that provide a direct mechanism to translate extracellular signals into transcriptional response from a cell.

In addition to the principal components of the pathway which is directly responsible for transcriptional response of the cell there are other proteins that have been identified that are involved in the function. Of these there is one class of effector proteins and another class of negative regulators that have been identified:

- **STAMs**: These are a class of effector proteins known as Signal Transducing Adapter Molecules (STAM). STAMs can also be phosphorylated by JAKs in the process of signaling. Although the mechanism of function of STAMs is less understood but they are known to facilitate the transcriptional activation of specific target genes.

- **StIPs**: These are another class of effector proteins known as Stat-Interacting Proteins (StIP). StIPs can associate themselves with both JAKs as well as unphosphorylated STATs and are assumed to facilitate the phosphorylation of STATs by JAKs.
• SH2B/Lnk/APS family: These are another class of effector protein adapters involved in the JAK-STAT pathway. Both SH2-Bb and APS associate with JAKs, but the SH2-Bb facilitates JAK/STAT signaling while the APS inhibits it. The contribution of the SH2B/Lnk/APS family to JAK/STAT signaling is not yet well understood like many other proteins that are associated, but it is clear that there exist various proteins outside the basic pathway machinery influence JAK/STAT signaling.

b. Scope of Hepatitis C

WHO estimates that about 170 million people, 3% of the world's population, are infected with the hepatitis C virus (HCV) and are at risk of developing liver cirrhosis and/or liver cancer. The transmission occurs through transmission of body fluids or by sharing of needles. The chronic form usually is asymptomatic, which makes clinical diagnosis difficult. The infection lasts for decades in individuals and the patient may or may not be aware of its presence which leads to a higher frequency of transmission. The disease remains asymptomatic till a certain level of progression. After which the virus causes severe irreparable damage to the liver and leads to cirrhosis or even liver cancer. This causes various symptoms that are can be mild to severe and include jaundice, inflammation and scarring of the liver, loss of appetite, IBS (Irritable bowel syndrome) and a series of gastrointestinal complications. The symptoms later have psychological implications like depression, moodswings, night sweats etc and are common in the chronic phase of the disease. The only option remaining at that stage is liver transplant, which does not ensure cure of the disease and often leads to the recurrence of the virus and/or death. According to the April 2004 HCV report from Decision Resources, there
are currently more than 10.5 million HCV positive individuals in the 7 major market countries viz. United States, France, Germany, Italy, Spain, United Kingdom and Japan.

<table>
<thead>
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<th>United States</th>
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<th>Japan</th>
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Decision Resources lists the following as market “drivers” for HCV therapies:

1. As the number of patients presenting with advanced liver disease and requiring treatment increases, the overall market will expand, the diagnosed population of patients who have been infected for more than 15 years will expand between 2005 and 2013.

2. Because the number of non-responding patients (those who do not respond to initial therapy) is increasing, HCV specialists are increasing their use of long-term maintenance therapies centered on interferon products.

3. Specialists are increasingly willing to initiate interferon therapy for patients with mild HCV infections and those co-infected with HIV.
4. A shift away from standard interferon products to more expensive PEG-Interferon products is expected, due to the improved efficacy provided by PEG-Interferons.

c. Current standards

Until the 1980s, interferon was scarce and expensive polypeptides. Today, with the use of Recombinant DNA technology, interferon is manufactured on a large scale through bacterial cultures. There are various companies manufacturing recombinant interferon alpha to address the problem of Hepatitis C and have products in the market as Pegasys (peginterferon alfa-2a) by Roche Pharmaceuticals, Pegintron (peginterferon alfa-2b) by Schering-Plough Corporation. The other non-pegylated interferons available in the market are Roferon (standard interferon alfa-2a) by Roche Pharmaceuticals and Intron A (Standard Interferon alfa-2b) from Schering-Plough Corporation. The current standard of cure is the administration of pegylated interferon alpha along with ribavirin. The treatment can be 48 to 72 weeks long depending on the viral strain. An aggravating factor in the prolonged antiviral treatment is the exposure to various side effects, which are suffered by all patients undergoing the combinational therapy. These side effects include flu-like symptoms, insomnia, leucopenia, thrombocytopenia, loss of appetite, loss of libido and many other neuropsychiatric effects. The greatest challenge facing clinicians and pharmaceutical companies who treat patients with chronic hepatitis C virus is maintaining full-dose therapy for the appropriate duration of time. Instances of common side effects which have a significant effect on the quality of life include fatigue, flu-like illness, anxiety, depression, irritability, insomnia, mood swings, loss of libido, loss of concentration, anemia, rash, joint pains, muscle aches and fever. The major side effects
being fatigue, pain, fever and severe depression, which are experienced by all patients. Furthermore, the overall 50% efficacy of the treatment still falls short of expectations. With the disease being asymptomatic in early stages, and the adverse and common side effects the treatment is often considered worse than the disease.

d. Proposition

With the developments in medicine in the past few decades, the consideration for the quality of life has moved to the forefront of human concern. Quality of life considerations include the ability to maintain a job, the ability to maintain personal relationships with friends and family, the ability to enjoy situations or events that previously gave pleasure and moreover the ability to continue to feel what it means to ‘live’ other than just being ‘alive’. The effects of Hepatitis C therapy are of important parameters because the disease is largely asymptomatic and the disease in its early stages seldom has any impact on patients' lives until the damage is done. These side effects can be attributed to a set to the mechanism of Interferon action. The systemically administered interferon is broadcasted all over the body to all the cells. With the help of JAK-STAT pathway the cells devote a significant effort of the cellular machinery into eliminating the viral infection. However the HCV infection is limited a much smaller number of the cells and is localized to the cells of the liver. The state of ‘alarm’ created by the interferon helps in knock down of the virus in the liver but also results in a cascade of side effects in the non-infected cells that do not need to be in a state of alarm. *The primary drug formulation discussed here is an interferon coupled with HDV® (Hepatocyte Directed Vehicle), a clinically proven hepatic targeting molecule and drug*
delivery system which has been used in to increase the efficacy of insulin by targeting to
the hepatocytes in the liver.

e. Conclusion

Thus it can be observed with the above mentioned information that there is are more patients that get infected with Hepatitis C and have a higher mortality rate than any other Hepatitis. The virus is highly mutative and exists in multiple subtypes and quasispecies, which makes developing a vaccine very difficult. With transfusion screening laws, regulations and recommendations from the government and CDC, in the United States, new cases of infection are consistently decreasing. However, there is a large population in USA as well as around the world that suffers from chronic Hepatitis C infection. The major reason of our inability to address the virus is the non specific symptoms in acute stages and early chronic stages. Thus many infected individuals remain undiagnosed and the probability of transmission is high. Another problem that exists with the therapy available for the treatment of HCV is the associated side effects of Interferon. It often is responsible for patients to drop out of therapy, which lasts for 28-48 weeks. There is an impending need to address this issue and to improve the tolerance of therapy in Hepatitis C patients.

4. Economics of the problem

According to the American Journal of Managed Care, HCV is the most common long-term blood-borne infection in the United States and is this country’s primary cause of liver transplants. The US Centers for Disease Control and Prevention estimate that 3.9 million (1.8%) Americans have been exposed to HCV. Because high-risk populations
such as prisoners, IV drug users, and the homeless are excluded from national surveys, such estimates of HCV prevalence are likely conservative. Approximately 20% of all HCV positive individuals will eventually suffer from cirrhosis of the liver. Most of the chronically infected are now in their 40s and 50s. As they continue to age, many of these individuals will be part of an increasing wave of HCV patients who experience mature clinical symptoms. At the same time, the number of new cases in the US has declined from an average of 240,000 per year in the 1980s to an average of 30,000 per year currently, largely due to improved blood donor screening and safer needle practices among IV drug users. The worldwide HCV population is estimated to be in excess of 150 million people.

The economic impact of chronic HCV infection in the US is expected to increase over the next ten years, despite the overall decrease in new HCV cases. This anticipated increase in the cost of the disease is attributable to the increasing number of HCV positive individuals who will experience higher levels of mortality and morbidity due to HCV cirrhosis and other complications. From 1992 to 1998, hospitalizations in which HCV-related liver disease was the either the primary or secondary reason for admission rose about 6-fold. Closely related to this is the finding that from 1990 to 2000 there was a 5-fold increase in liver transplants in HCV positive recipients.

Perhaps the most significant impact of a new drug formulation could be, to provide interferon therapy that can be administered chronically with minimal side effects and improved efficacy. It is estimated that only 50% of HCV patients respond to traditional interferon therapies, leaving a significant percentage of the population without meaningful therapeutic alternatives. It is hypothesized that this low level of response is
due, in part at least, to the lack of delivery of significant concentrations of interferon to infected hepatocytes. The ability to provide a non-injectable formulation could also provide an important improvement in dose administration for HCV patients.

5. Approach

a. Evolution of HCV Therapies

It is improbable to determine the origins of the Hepatitis C virus since there are no records of stored samples of blood older than 50 years and the virus has been identified in the recent years so there are no confirmed records of patients suffering from HCV infection in history. However like most viruses HCV, is found in remote areas all over the world in closely related strains, hence it can be concluded that Hepatitis C virus has probably been around for thousands of years before evolving into current strains.

In the 1970s Harvey J. Alter’s work at the Infectious Disease Section at the Department of Transfusion Medicine at the NIH concluded that most post transfusion hepatitis infections were not Hepatitis A or B (NANBH- Non A,Non B Hepatitis). Later in 1988, Alter confirmed the existence of a new species in a panel of
NANBH samples. In April of 1989, the discovery of the virus, re-named hepatitis C virus (HCV), was published in two articles in the scientific journal ‘Science’ \(^{xxx, xxxi}\). Later in 1992 the confirmatory tests for HCV were applied to blood transfusion supply and organ were screened for donations. This has led to a significant reduction in the risk of contracting Hepatitis C through blood transfusion over the years and is as low as 0.01\(^{xxxii}\).

b. Innovation

The evolution of HCV therapies has cleared a path for interferons. These were in-turn developed on the lines to increase their circulation time in the body. In 2001 The PEGylated Interferon alpha 2b (Peg-Intron\textsuperscript{®}) from Schering Plough got FDA approval and was introduced into the market for the treatment of Hepatitis C. Peg-Intron is a powder that needs to be reconstituted before it can be injected. Today, Peg-Intron is now available in “Redipen” for dosing and reconstitution. Pegylation brought about a new era in the treatment of Hepatitis C. The standard protocol for therapy was to administer the interferon 3 times a week. The body broke down the previously available synthetic interferon rapidly within 12 to 24 hours after injection. Once the synthetic interferon was broken down in the body there is no interferon available to suppress the viral infection till the next dose was administered. PEGylation is the addition of PEG (Poly Ethylene Glycol) to the surface of the interferon. PEG was essentially a polymer of Ethylene Oxide and has a characteristic of preventing fast clearance of a protein from the blood if attached to its surface. This increased the circulation time of the protein which in-turn provided improved effects and

\[ \text{Figure 5: Poly Ethylene Glycol Structure} \]
reduced toxicity. With increased circulation time it also enabled reduced dosage and longer intervals for administering the protein.

PEGylation brought significant improvement in the administration and bioavailability of the interferon for the treatment of Hepatitis C. In the past years, after the introduction of PEGylation, there has been a significant effort to develop other masking technologies (similar to PEG) to increase the circulation time in blood. The use of other molecules to augment in combinational therapy with Interferon is also being researched. Both these lines of research have a scientific promise and intend to increased efficacy in treating various strains of Hepatitis C virus. However the treatment of Hepatitis C has a strong social aspect that cannot be ignored. The standard treatment duration is from 48-72 weeks. Most patients tend to drop out of therapy due to the side effects of the combinational therapy, and primarily the interferon. With increased circulation time or with multiple molecules augmenting the interferon in combinational therapy, the side effects of interferon cannot be ignored. Very little effort has been put in improving the life standard of the patients suffering from Hepatitis C that undergo treatment. There is a pressing need to address the adverse effects of interferon to ensure and improve patient compliance to the therapy.

c. Competitive Landscape

Competitive landscape for HCV therapies under development:

• **Interferons:**
These include drugs under development that can be alternatives to the standard mode of treatment. Potential substitutes for PEG Interferon:

Locteron by BiolexTherapeutics / OctoPlus: Alpha interferon, using Polyactive Technology the formulation is aimed at reduction in dosage frequency by sustained release mechanism for Interferon. The proposed breakthrough is in-line with use of PEGylation for increased circulation time with reduced frequency of dosage. Locteron is designed to be administered by subcutaneous injection.

Omega Interferon (OI) by Intarcia Therapeutics: OI is a new type of interferon to be introduced for the treatment of Hepatitis C and uses a mechanical pump to maintain levels of interferon in the blood. Recent tests on subjects suggest that OI attains efficacy comparable to Pegylated alpha interferon. OI is designed to be administered subcutaneously.

Multiferon (Long acting) by Viragen: Approved for certain types of Melanomas, however no clinical trials for the study of efficacy have been announced in the United States. It is a combination of interferons and the therapy is based on natural formulation of alpha interferon.

Albuferon (Long acting) by HGS: Alpha interferon therapy using albumin-masking technology. Technology focused on reduction in frequency of dosage. In-line with use of PEGylation for increased circulation time with reduced frequency of dosage. Albuferon is projected to enter the market in 2010. High dosage toxicity issues to be addressed. To be injected subcutaneously.

• Non Interferon Therapies
**Nucleosides:**

Non interferon therapies under development are alternatives to the current standard mode of treatment. They provide a strong promise to non responding patients and possibly to reduce the duration of therapy for other patients. However they need to be administered along with interferon as a part of the combinational therapy and do not pose a direct threat to interferon and interferon based therapies in the near future. Depending on proven formulation, efficacies and functional pathways they might act as potential substitutes of Ribavirin.

Thus nucleosides do not pose a direct threat to interferon therapies and other therapies that use interferon like Hepasome’s HDV interferon. In light of the modes of administration of HDV-IFN, these nucleosides can be used along with HDV-IFN to improve viral response in patients.

**Anti Inflammatory:**

These are a class of formulations that do not promise to cure the Hep C infection in any way. Liver being one of the most important and busy organs of the human body suffers severe damage due to the infection. This damage has various effects that may or may not be permanent. The Anti-Inflammatory drugs being developed for Hep C infected patients are to address these damages and/or to minimize the discomfort that they may cause the patient. It is important to notice that an entire class of formulations are being developed to improve the life standard of the patient due to the significant mental and psychological impact of Hep C infection as well as the side effects of the interferon therapy.
Since these drugs do not cure or attempt to cure Hep C infection, they need to be administered along with Interferon or Interferon based therapies. They do not pose any threat to interferon based therapies. They are to be administered along with the interferon based therapeutics to prevent and reduce any secondary complications of the infection as well as any permanent damage the virus may cause.

**Viral Pathway Inhibitors:**

Viral pathway inhibitors are a class of new compounds well known for their antiviral promise that they have shown in other viral diseases like AIDS. However significant research needs is yet to be done to address their behavior and toxicity for treatment of Hepatitis C infection. These are suggested to assist in viral knock down but have many cyto-toxic issues associated with them. They do not pose a direct threat to interferon and interferon based therapies for two major reasons that are, significant associated side effects and inability to attain sustained viral response without interferon therapy.

Since these drugs are being developed to be used in combination with interferon, they do not pose a direct threat to interferon or HDV-IFN in the near future. However it cannot be ignored that in the near future possible Viral Pathway inhibitors can be developed that may show sustained viral knockdown without interferon.

d. Proposition

Understanding the demand for addressing the adverse effects of interferon is a must. However achieving higher efficacies is also important, especially for non responding patients who have failed to respond to the standard Interferon therapy. The theory
underlying this proposition is to localize the interferon into the liver. Since the highest damage and activity of the virus is within the liver, if a limited dosage of interferon is restricted to act within the liver, the side effects that are witnessed all over the body due to interferon can be significantly reduced. With the localization of interferon in the liver higher concentrations can be achieved to get higher viral knockdown. This is currently difficult to attain because the side effects of interferon limit the dosage that can be administered to the patient.

Thus a delivery mechanism that can limit the interferon activity to the liver can substantially increase viral knockdown and at the same time reduce the systemic side effects of interferon administration.

e. Current technologies and pipeline

The market for providing improved therapies for HCV has tremendous potential in the US and abroad. Because HDV-INTERFERON has the potential to improve the efficacy of interferon and to decrease the overall toxicity normally associated with interferon therapy, Hepasome is optimistic that its HDV-INTERFERON therapy will move expeditiously through the clinical trial process. With respect to the oral form of HDV-INTERFERON, Hepasome believes that recent clinical data from an HDV application of targeted insulin in diabetes patients provides a compelling rationale for the applicability and utility of this technology for oral interferon.

Decision Resources estimates the total market for HCV therapies to reach $10.7 billion per year by 2013. These projections are based in part upon estimates that the worldwide HCV population is continuing to expand and that the total number of HCV patients in 2013 could exceed 10 million worldwide. Further, many of these patients will
not respond to current first-line therapeutics, thereby providing a significant opportunity for improved, liver-specific forms of therapy.

Following is a list of therapies that are currently under pipeline in various companies. They use various pathways to address the demand for a better therapeutic formulation to address the problem of Hepatitis C infection.

<table>
<thead>
<tr>
<th>Product Under Development</th>
<th>Mode of Function</th>
<th>Company</th>
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<tbody>
<tr>
<td><strong>Nucleoside</strong></td>
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<tr>
<td><strong>Viramide (Taribavirin)</strong></td>
<td>Nucleoside Analogue</td>
<td>Valeant Pharmaceuticals Int’l</td>
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<td></td>
<td></td>
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<tr>
<td>A pro-drug of ribavirin that specially targets the liver. VISER Clinical studies focused on reduction of anemia caused due to nucleoside activity of Ribavirin. Phase IIa data displayed loss of efficacy but substantial reduction in anemia.</td>
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<tr>
<th><strong>Anti-inflammatory</strong></th>
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<tr>
<td><strong>JBK-122</strong></td>
<td>Anti-inflammatory</td>
<td>Jenken Biosciences</td>
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<tr>
<td>The drug received FDA approval for Phase II clinical studies as announced in December 2006. The goal of the study was to prevent liver damage caused due to HCV related inflammation. No further data or updates have been announced.</td>
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<tr>
<td><strong>CTS-1027</strong></td>
<td>Anti-inflammatory</td>
<td><strong>Conatus</strong></td>
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<tr>
<td>The company announced initiation of Phase II clinical trials in December 2007. The goal of the study is to prevent and possibly reduce liver inflammation due to alcohol, obesity, autoimmune responses and viral infections like Hepatitis C.</td>
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<tr>
<th><strong>Zadaxin (Thymalfasin)</strong></th>
<th>Immunomodulator</th>
<th><strong>SciClone/Sigma –Tau</strong></th>
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<tbody>
<tr>
<td>Data from the Phase III trial expected to be publicly announced by year end 2008. The drug focuses on boosting immune response in the human body without any additional side effects. Administered along with the standard mode of treatment.</td>
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**Viral Pathway Inhibitors**

<table>
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<tr>
<th><strong>MX-3253 (Ceglosivir)</strong></th>
<th>Glucosidase I Inhibitor</th>
<th><strong>Migenix</strong></th>
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<tr>
<td>Additional drug formulation designed to be used in conjunction with the standard mode of treatment.</td>
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<tr>
<th><strong>VCH-759</strong></th>
<th>Polymerase</th>
<th><strong>Virochem</strong></th>
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<td>Additional drug formulation designed to be used in conjunction with the standard mode of treatment.</td>
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<tr>
<th><strong>R1626</strong></th>
<th>Polymerase</th>
<th><strong>Roche</strong></th>
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<tr>
<td>Additional drug formulation designed to be used in conjunction with the standard mode of treatment.</td>
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The biopharmaceutical industry is characterized by extensive research efforts and rapid and significant technological change. In the development and marketing of prescription drugs, Hepasome faces intense competition. The competitive landscape for HDV-INTERFERON includes Interferon and other antiviral therapies. Currently Pegylated-Interferon with Ribavirin administered as combination therapy is the standard of care. Frost and Sullivan, in January 2006, stated “The key competitive factors in the hepatitis C market include efficacy, convenience of dosing, side-effect profile, and efficacy in Interferon with Ribavirin therapy-failed patients.”

<table>
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<tr>
<th>SCH 503034</th>
<th>Serine Protease</th>
<th>Schering</th>
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<td>Additional drug formulation designed to be used in conjunction with the standard mode of treatment.</td>
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<tr>
<th>VX 950</th>
<th>Protease</th>
<th>Vertex</th>
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<td>Additional drug formulation designed to be used in conjunction with the standard mode of treatment.</td>
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<tr>
<th>TMC435350</th>
<th>Protease</th>
<th>Medivir / Tibotec</th>
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<tr>
<td>Additional drug formulation designed to be used in conjunction with the standard mode of treatment.</td>
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<tr>
<td>Product Under Development</td>
<td>Type of Interferon / Clinical Phase</td>
<td>Company</td>
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<tr>
<td>Locteron</td>
<td>Alpha interferon, Phase II</td>
<td><strong>Biolex Therapeutics</strong></td>
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<td><strong>/OctoPlus</strong></td>
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Using *Polyactive* technology from Octoplus with Biolex’s human alpha interferon, the resultant is a slow release long acting interferon. The Phase IIa trial established comparable results of Locteron with respect to Pegylated Interferon currently available in the market.

| Omega Interferon          | Omega interferon, Phase II         | **Intarcia Therapeutics** |

The Phase 2 trial attained efficiencies compared to Pegylated Interferon+ Ribavirin therapy (SVR 52%-63%) formulations currently available in the market.

Administration is through the use of “DUROS” device as a pump to achieve consistent blood interferon levels.

| Multiferon (Long acting)  | Natural Alpha Interferon; Phase II | **Viragen**              |
Available in many countries other than United States for the treatment of HCV. In Vitro studies conducted on cell lines by U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) in early 2006. No clinical trials announced in the US (September 2007).

<table>
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<tr>
<th>Albuferon (Long acting)</th>
<th>Alpha Interferon, Phase III</th>
<th>Human Genome Sciences</th>
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Dosage (with Ribavirin) every two and four weeks, attained SVR 55.5% and 50.9% as compared to 57.9% for Pegasys (with Ribavirin). Attained overall SVR in non-responders of 17.4%. Adverse issues associated as pulmonary events. January 2008, during Phase III trials 1,200mcg dosing arm discontinued due to adverse events. HGS expects clinical trial to commence and file for marketing approval by 2009-10.

6. Solution:

a. The HDV Technology

HDV is a platform nanotechnology specifically designed to address the inability of current formulations to target Hepatocytes, which as a therapy for treatment of HCV translates as the inability of administered human interferon to be targeted to the liver, thus limiting the immunomodulatory effects, resulting in reduction of unfavorable systemic side effects. HDV-INTERFERON is composed of nano-sized membrane vesicles composed of recognized substances (such as distearoyl lecithin, cholesterol and dicetyl phosphate) and a hepatocyte-target molecule with a specificity for hepatocytes. The HDV delivery system is comprised of very small (> 50 nanometers in diameter) lipid
vesicles or fragments. **These vesicles or fragments contain a simple, yet sophisticated, proprietary targeting molecule that is specifically pulled from the blood by receptors on the surface of hepatocytes in the liver.**

The small size of liposomes enables them to be used as oral drug formulations. The small size of the particles make them stable and enable passage through the walls of gut into the hepatic portal vein (<150nm liposomes) xxxiii. Delivery of interferon to hepatocytes via HDV-INTERFERON should not be confused with the conventional delivery of hydrophobic drugs or proteins by liposomes. Conventional liposomal delivery of drugs is based on the bulk transport of a poorly available drug substance by prolonging its transport in the blood within single or multi-lamellar lipid vesicles of various sizes. In contrast, the HDV targeting technology very rapidly delivers efficacious quantities of interferon to hepatocytes with high efficiency.

The very small (nano) size (see Figure.1) of the vesicles imparts a number of advantageous characteristics to the delivery system, including:

Survival *in vivo* because the particles are much too small to be removed by macrophages, including Kupffer cells of the liver, in contrast to significant catabolic elimination of typical standard formulations of liposomes or multi-lamellar vesicles by these same cells. HDV-INTERFERON has a very short circulating half-life in blood due to its hepatic targeting molecule.

Like very small viral particles (~50nm) the nano-sized membrane vesicles are small enough to enter the circulatory system from the intestine between
the absorptive cells, thus contributing to the oral physiologic activity of HDV-INTERFERON vesicles.

Originally, the Hepatocyte Directed Vesicles was developed and tested (animal and human) with a chromium (bis)-disofenin Hepatocyte targeting molecule (HTM). Recent studies with the biotin phosphatidylethanolamine as the HTM (animal and human) has shown advantages over the chromium (bis)-disofenin. The Hepatocyte Delivery Vesicles with the biotin has many advantages over the chromium (bis)-disofenin. They are:

- Biotin is a USP vitamin found in one a day tablets at 30 mcg, which is more than 50 fold higher than would be delivered to a patient by a typical daily dose of the Hepatocyte Delivery Vesicles.
- Phosphatidylethanolamine is a natural lecithin found in abundance in the body. For example, it is about 23% of the mass weight of gray matter in the brain. (PE) is a lecithin covered by USPNF 23.
- Biotin is linked to PE by an amide linkage which is hydrolyzed by enzymes in the liver.
- Biotin-PE is a more effective Hepatocyte Targeting Molecule than chromium (bis)-disofenin by effecting normalization of hepatic glycogen in rats by 1 hour post dosing compared to 2 hours for the chromium (bis)-disofenin as shown in Bioassay of Hepatic Targeted Insulin: Liver Glucose Uptake in Diabetic Rats study. Biotin-PE is also more effective than chromium (bis)-disofenin as a target molecule for HDV delivery of interferon alfa to the liver, as measured by liver PKR levels with both oral and subcutaneous dosing in normal mice.
- There is no chromium in Biotin-PE.
Toxicological Comparison of Biotin-PE with Chromium (bis)-disofenin

- Biotin is a USP vitamin which is included at a dose of 30 mcg in most multiple vitamin products. It is required by the liver for normal metabolism.
- Phosphatidylethanolamine is a major, normal constituent of the body, making up to 23% of the gray matter in the brain. It is a lecithin described in USPNF 23.
- Chromium (bis)-disofenin, as the Hepatocyte Targeting Molecule component of the current Hepatocyte Delivery Vesicles, has been shown to have no adverse toxicity in a 28 day rat study when administered subcutaneously in doses up to 2.5 mg/kg body weight/day. In addition, HDV with chromium (bis)-disofenin as the hepatocyte target molecule, has been subjected to the mouse micronucleus assay and the mammalian chromosome aberration test at BioReliance, Rockville, Maryland. These studies have been completed and no test material-related adverse effects were found.
In a formal meeting with the MHRA (Britain) in July 2006 (proposed rapteur country for the EU for HDV-Insulin), the Health Authority concurred with the proposed change in HTM molecule and indicated that no additional toxicology studies would be required for short or long term human trials. In addition, during an End-of-Phase II Meeting with FDA in March 2007, the HDV system with biotin was approved for long term testing (Endocrine Division).

b. Mechanism: How Does HDV-INTERFERON Work?

Subcutaneous administration of HDV-INTERFERON vesicles provides a directed supply of interferon to the liver directly. The HDV delivers interferon directly to the liver and significantly reduces interferon levels in the peripheral organs and tissue like the spleen, thus reducing undesirable systemic side effects.
Oral HDV-INTERFERON nano-vesicles are stable at low pH and in blood and are small enough to:

- Avoid sequestration by macrophages,
- Be protected against enzymatic degradation, and
- Penetrate between absorptive cells in the intestine.

Once in the blood, HDV-INTERFERON particles will be rapidly removed in first pass clearance by the liver.

c. The Potential Benefits:

- Chronic hepatitis care is sub-optimal, creating opportunities for new technologies that deliver substantial improvements in sustained viral reduction.

- Clinical endpoints for interferon therapies are well-characterized and understood by regulatory agencies, clinicians and patients.

- Because interferon side effects limit the drug’s efficacy to 50% or less, a liver targeted interferon that improves patient compliance and reduces side effects could have a major impact in disease care.

- Injectable and Oral HDV-Insulin (by Diasome, using the same HDV technology) have already demonstrated significant efficacy in human patients, thereby lowering the development risk profile of HDV-INTERFERON formulations.
• HDV-INTERFERON improvements can be demonstrated in relatively few patients and at a low cost.

• HDV-INTERFERON is compatible with all commercially available forms of human interferon.

• Current oral interferon technologies under development have not solved the problem of low bioavailability; using the same technology for solving the bioavailability problem with oral insulin, pre-clinical data indicate that Oral HDV-INTERFERON has bioavailability that is similar to injected HDV-INTERFERON.

• Oral HDV-INTERFERON could provide HCV patients with a non-invasive alternative.

• Existing intellectual property and applications covering broad aspects of the product, technology and manufacturing process.

• HDV-INTERFERON is manufactured with readily-available materials and equipment, at a low cost of goods.

• Upon completion of Phase II studies, HDV-INTERFERON could be the first biopharmaceutical formulation in oral interferon therapy.

7. Development

All pharmaceutical products need to undergo a process of rigorous testing in aspects of safety and efficacy in animals and later in humans. Clinical trials involving new drugs and formulations like HDV-IFN can be classified into four phases. The sponsoring company of a clinical trial has to seek the approval of the Food and Drug
Administration (FDA) for each trial before they can proceed to the next and hence each trial is treated as a separate clinical trial. After the drug has successfully passed through the Phases I, II, and III, it is usually declared safe for use in general population and approved to enter the market. Phase IV are studies involved 'post-approval' by the regulatory authorities to study other aspects of the drug like long term exposure, responses in specific populations like pregnant women. Phase IV studies can also be pursued by a company for competitive reasons or other indications.

a. Pre-Clinical:

i. Proof of concept studies: (Phase 1)

A variety of pre-clinical experiments have been conducted on both HDV and HDV in combination with proteins, administered by injection or the oral route. In one of the earliest studies, 5nm gold particles were encapsulated in non-targeted control vesicles and were administered intravenously to intact mice. The non-targeted vesicles were found only in Kupffer cells whereas with **HDV targeted vesicles, the gold particles were found only in hepatocytes confirming its targeting abilities.**

ii. Bio-distribution

The HDV liposomes were labeled with 14C-cholesterol placed in the liposome membrane to trace the fate of HDV. A bolus intravenous (IV) dose of 14C-labelled HDV was given to mice and samples of blood, spleen, and liver were analyzed for radioactivity at each time point.

Radio labeled HDV rapidly disappeared from blood and rapidly appeared in the liver, with approximately 80% of the radioactivity found in the liver within 60 minutes of
administration. At 240 minutes after administration, approximately 85% of the radioactivity was observed in the liver, approximately 10% in the blood, and approximately 5% in the spleen. Recovery of radioactivity was essentially 100%. Similar results were obtained after administration of radiolabeled HDV with the added insulin. The results of this study confirmed that the distribution of HDV is targeted to the liver.

iii. Oral route, Efficacy

Experiment in rat to demonstrate Gut Absorption of non–targeted Oral Liposomes

Non-targeted and non-RES masked Oral liposomes were labeled with 14C phospholipid in the liposome membrane. Following filtration through a micro filter, the mean particle size of the Oral Liposomes was less than 100 nanometers as measured with a Coulter Sub-micron Particle Size Analyzer. A 10 mg/kg body weight sample containing radio-labeled liposomes was injected into the duodenum of an anesthetized fasted, but otherwise normal, rat. Blood was taken from the portal and femoral veins at 15 and 30 minutes post-dosing for counting, later the rat was sacrificed, and samples of blood, liver, and spleen were removed for counting. It was found that oral liposomes, as measured as 14C, were found in both portal and femoral blood. The portal blood levels were higher than the femoral blood as expected (Figure 2). Approximately 2% of the liposomes were found in the liver and about 1% was found in the spleen. Considering the relative sizes of the liver and spleen, the splenic uptake was much higher on a weight basis as would be expected for non-RES masked liposomes on a weight basis as would be expected for non-RES masked liposomes.
Thus it was found that oral liposomes can cross the gut wall into the blood stream and are candidates for oral drug delivery of materials that otherwise do not cross the gut wall into the blood stream.

Figure 7: Levels of orally consumed liposomes, stating the possibility of usage of liposomes as candidates for oral drug delivery of materials across the gut wall into the blood.

Figure 8: Levels of distribution of HDV insulin in Blood, Liver and Spleen in the body.
iv. **Hepatic Targeting**

To demonstrate the absorption of HDV from the gut, 14C HDV liposomes were administered to fasted mice via their drinking water. HDV was absorbed from the gut in mice that had ingested water with radio labeled HDV. In Figure 4, approximately 8% of the ingested dose of HDV was found in blood by 15 minutes after the water had been removed from the cage. The amount in blood remained constant between 15 and 45 minutes. The liver uptake was about 8% at 45 minutes and the spleen was approximately 1% of the ingested dose (Figure 5). The total absorption was approximately 17% (total of blood, liver and spleen).

It is important to contrast the data from this experiment with the data from the first study. The absorption of the non-targeted liposomes totaled 17% at 30 minutes post dosing. The absorption of the targeted HDV liposomes totaled 18% at 30 minutes. The difference is in the distribution: the liver contained only 2% of the non-targeted liposomes, but the HDV, liver targeted liposomes had a liver distribution of 8%. The blood level in the HDV group was half that of the non-targeted liposomes, indicating the specificity of the targeting mechanism. Hence, the conclusion that can be reached is although the overall gut absorption was the same for targeted and non-targeted liposomes. The difference in distribution depends on targeting.

- *The liver contained only 2% of the non-targeted liposomes, but targeted liposomes had a liver distribution of 8% which implies that 1/4th dosage of HDV-IFN is required to get the same viral knockdown as normal Interferon.*
- *HDV has been shown to cross from the lumen of the gut to the portal and peripheral blood in mice, which implies that the interferon can be administered*
orally to the subjects reducing reactions at site of infections which is a common side effect of normal interferon injections.

Figure 9: Blood levels of HDV orally ingested with drinking water in mice.

Figure 10: Levels of uptake of HDV, distribution different organs of the body after orally ingesting with drinking water, in mouse model.
v. Pharmacology

HDV-Interferon Alpha effect on PKR in Mice

Previously performed pilot animal experiments, test the validity of the HDV delivery hypothesis. Using 2 groups of mice (test and control) the test group received HDV-INTERFERON with chromium (bis)-disofenin as the HTM, while the control group received interferon alone. The induction of the double stranded RNA dependent protein kinase (PKR) gene was used as a marker of interferon tissue delivery.

The assay used was real time quantitative PCR (polymerase chain reaction) to assay the level of PKR messenger ribonucleic acid (mRNA). The spleen was used as a surrogate for systemic delivery of the marker and compared it to hepatic delivery. After 4 hours, a 4-fold switch in the PKR expression level from the spleen to the liver was observed, i.e. the HDV caused the interferon to be delivered more effectively to liver cells than to

![Figure 11: Effect of HDV targeted interferon in liver compared to interferon alone](image-url)
spleen cells. We believe that testing the PKR expression levels after 12 or 24 hours will show even more significant response.

HDV-INTERFERON with biotin-PE as the HTM has also been studied in mice. The study was designed to determine the effect of interferon on PKR levels in liver. PKR is an RNA marker responsive to interferon alpha. Subcutaneous control interferon was compared to subcutaneous HDV-INTERFERON and Oral HDV-INTERFERON. The PKR responses were similar for both routes of HDV-INTERFERON administration and both were superior to the PKR response of subcutaneous control interferon alpha. All doses were the same, and were at the same per kilogram dosage as the human 3,000,000 IU dose.

![Figure 12: Effect of HDV Targeting on cells of the liver in mice](image-url)
vi. **Toxicology Studies**

The initial targeting vesicle formulation (HDV carrier without interferon attached) was tested in male and female mice by subcutaneous doses of 0 (control, vehicle only), 0.6, 1.2, or 2.4 mg/kg/day for 28 days. The highest dose level was selected to provide a safety factor of 2,400 times the projected dosage in the initially planned clinical trial. No serious safety concerns were observed. Some mild inflammation at injection sites and in lymph nodes was noted and was considered to be consistent with subcutaneous injections. A series of genotoxicity studies have been completed to test the HDV carrier. In the Ames Assay, no reverse mutations were induced; nor was HDV found to be mutagenic nor genotoxic in the In Vitro Mammalian Chromosome Aberration Test and a Mouse Micronucleus Test. The FDA has confirmed that no additional toxicity studies for HDV using the initial targeting agent are required for NDA submission for the HDV-Insulin development program in the US.

The second HDV formulation contains a naturally occurring substance as the hepatic targeting agent and is itself used at a dose far below the level of normal dietary intake. Based on these facts and the demonstrated safety and tolerability of the lipid vesicle preparation, as well as those of approved liposomal products, We consider that no additional pre-clinical safety/toxicology studies are needed.

**b. Clinical development:**

Hepatocyte Directed Vesicles ("HDV") are nano-carriers comprised of amphipathic phospholipids. HDV + human insulin is currently in human clinical trials as a hepatic delivery vehicle for insulin. Clinical data from several studies have shown
impressive enhancements of hepatic glucose metabolism in diabetes mellitus patients, with no treatment associated adverse reactions.

We postulate that targeting interferon alpha to the liver will increase its efficacy and reduce therapy-related side effects. It is also possible that HDV targeting of interferon will also lower the required dose to achieve efficacy. And, delivery of interferon to the liver may also overcome the resistance to therapy that has been observed in the past.

i. Clinical Safety

All the Phase I and Phase II trials to date with the HDV carrier system indicate it was well tolerated and appeared to be safe at the doses studied. None of the out-of-range laboratory values was determined to be clinically significant or drug related. No time-dependent or concentration-dependent changes in laboratory parameters or vital signs were observed.

Overall Conclusion: *HDV as a targeted carrier has been demonstrated the safe and effective hepatic targeting of oral and subcutaneous proteins.*

ii. Clinical Trials:

Current interferon therapy plays a key therapeutic role in treating the growing Hepatitis C population. Its sub-optimal efficacy and unfavorable side-effect profile allows for developing advanced interferon products that may improve efficacy while limiting side-effects. In the Phase IIa study of HDV-INTERFERON, the Company is evaluating the initial efficacy and safety using the new oral route of administration in
naïve patients along with improved efficacy in a difficult-to-treat non-responder population with the new injectable (subcutaneous) formulation. As the duration of treatment will be 24 to 48 weeks dependent on their viral genotype, it is important to initially demonstrate the early viral load reduction within the first 4 weeks of treatment. A larger Phase IIb study will be performed in late 2008 after the establishment of the successes of Phase IIa study. Before conducting the Phase IIb trial, We will conduct an oral gelatin capsule bridging study that shows the liquid formulation and the capsules to be equivalent.

iii. Tranche vise clinical trials for risk mitigation

Clinical trials cost are high and there is a significant risk involved in response to execution as well as the human response to the formulation. One strategy that was used by Hepasome Inc. for the Phase II clinical trial was offering a tranche vise investment. The clinical trials were split into a 2 smaller trials viz. Phase 2A and 2B. The funding required for Phase 2A is significantly lower than the entire cost of Phase 2. This offers benefits of financial flexibility and lower risk profiles as compared to a large investment for the entire Phase 2 trial.

1. Phase 2A: Phase IIa (“Initial part”) - 4 weeks of treatment (28 days): This part shall assess whether the initial 4-week treatment course with HDV-INTERFERON, orally or by subcutaneous injection, and weight based ribavirin results similar efficacy [Rapid Virologic Response (RVR)] and safety as the reported efficacy and safety with pegylated alpha-interferon-2a and ribavirin (historical control) in patients with chronic hepatitis C (treatment naïve and non-responders).
Part IIb (“Continuation part”) – 20 or 44 weeks of treatment + 24 weeks (follow-up period):

1. Patients who achieve RVR will be treated for another 20 or 44 weeks based on their genotypes followed by 24 weeks of treatment-free follow-up period.
   - Patients, who do not exhibit RVR but achieve a drop in the HCV RNA levels at Week 4 may be allowed to remain in the study for further treatment and evaluation.
   - Patients, who achieve partial/complete EVR (Early Virological Response) at Week 12, will receive study treatment for an additional period of 36 or 12 weeks (to complete the duration of total treatment) depending on their viral genotype.

Follow-Up Period:

- **Follow-up period (24 weeks):** Thus, in addition to treatment parts, each completed patient will have 24 weeks of treatment-free follow-up.

Overall Study Duration (72 or 48 weeks):

Patients with viral genotype 1 will have overall study duration of 72 weeks (48 weeks of therapy plus 24 weeks follow-up) and patients with viral genotype 3 will have an overall study duration of 48 weeks (24 weeks of therapy plus 24 weeks follow-up). HDV combined with interferon has not been tested in humans. However, the same carrier system has been administered in several clinical trials with insulin under IND #56, 596, by injection and oral routes of delivery with demonstrated efficacy and no safety related effects, either clinically or in laboratory values. This HDV Carrier with insulin has been cleared with Phase III testing by the US FDA.
Potential Risks and Benefits

In non-clinical studies performed to evaluate the pharmacological properties of the current HDV product and in a 28-day toxicity study with the original HDV in rats, which included histopathological examination of the liver, there were no observations reported to suggest a serious safety concern with the HDV component of the product. In the toxicity study, an inflammatory reaction was noted in the lymph nodes and at injection sites which was regarded as consistent with a response to subcutaneous injections. No animal reproductive studies have been performed with the product. (Note: The US FDA has indicated, in writing, that neither reproductive nor long-term toxicity studies are required with HDV.)xxxvii There have been no drug related-adverse events in any clinical trial performed to date using HDV as the carrier.

Although no adverse reactions are anticipated, because of the limited experience with the HDV component in humans, the investigator should be alert to any possible indication of an adverse reaction including those events, which might be expected in conventional interferon therapy as well as allergic responses to any component of HDV.

2. Phase 2B

Based upon the initial data obtained from the Phase IIa trial, a Phase IIb study will be commenced. As noted earlier, we anticipate that we will have both 4 week and 12 week data from most of the patients by late 3Q2008, allowing the study plans to be finalized and the Phase IIb trial launched in late 2008.

This study is tentatively called “A Phase II B Trial of Two Dose Routes of HDV-Interferon (HDV-INTERFERON) Administered with Ribavirin in the Treatment of
Chronic Hepatitis C Non-responders and Naïve Hepatitis C Patients.” It will be a double-blind, multicenter (up to 40 study centers) and 200 completers (40 per group). The study hypothesis to be tested is whether treatment with HDV-Interferon (HDV-INTERFERON), by oral or subcutaneous (injection) routes, and ribavirin results in improved (injection) or non-inferior (oral) efficacy [Rapid Virologic Response (RVR)] and safety compared with pegylated alpha-interferon-2a and ribavirin in patients with chronic hepatitis C (for both treatment naïve and non-responders groups).

Following this strategy, We will have RVR and EVR data in the 2009 and SVR data in early 2010. We will seek a development partner during 2009 – 2010 to continue the Phase III late stage development of HDV-INTERFERON.

iv. General considerations for Clinical Trials

- Subject Information and Informed Consent

Informed consent and subject information should be given by means of a standard written statement, written in non-technical language; must be prepared in accordance with ICH E6 Section 3 and all applicable local regulations and approved by the IRB/IEC/EC. The subject must understand the statement before signing and dating it and should be given a copy of the signed document. No subject can enter the study before his/her informed consent has been obtained.

1. Discontinuation of the Study

Hepasome Pharmaceuticals reserves the right to discontinue this study for safety or administrative reasons at any time. Should the study be terminated and/or the site closed
for whatever reason, all documentation and study medication pertaining to the study must be returned to the Sponsor or its representative.

2. Changes to the Protocol

This protocol cannot be altered or changed except through a formal protocol amendment, which requires the written approval of Hepasome Pharmaceuticals. The protocol amendment must be signed by the investigator and approved by the IRB/IEC/EC before it may be implemented. Protocol amendments will be filed with the appropriate regulatory agency (ies) having jurisdiction over the conduct of the study.

3. Adherence to the Protocol

There are to be no deviations/violations from this protocol without the approval of the Sponsor. Any deviation/violation must be approved and must be in writing and signed by the Sponsor. The Investigator and research team must comply with all applicable local laws. Deviations from the inclusion or exclusion criteria are permitted by written permission from the Hepasome Medical Monitor prior to entry of the subject into the study. In such instances, a letter verifying the deviation from the inclusion or exclusion criteria will be sent to the site and will be placed in the study file.

4. Use of Information and Publication

All information concerning HDV-IFN, operations of Hepasome Pharmaceuticals, patent applications, formulae, manufacturing processes, basic scientific data, and formulation information supplied by Hepasome Pharmaceuticals to the investigator and not previously published, is considered confidential and remains the sole property of Hepasome Pharmaceuticals. Case report forms also remain the property of Hepasome.
Pharmaceuticals. The investigator agrees to use this information only to accomplish this study and will not use it for other purposes without written consent of Hepasome Pharmaceuticals. The information obtained in this study will be used by Hepasome Pharmaceuticals in connection with the continued development of HDV-IFN and thus may be disclosed as required to other clinical investigator(s) or government regulatory agency (ies). Publication or other public presentation of results from this study requires prior review and written approval of Hepasome Pharmaceuticals. Abstracts, manuscripts, and presentation materials should be provided to Hepasome Pharmaceuticals for review at least 30 days prior to the relevant submission deadline.

5. **Regulatory**

We will develop up to two distinct drug products in the application of HDV technology to interferon therapy: Oral HDV-INTERFERON and Injectable HDV-INTERFERON for subcutaneous administration.

The initial Phase IIa trial is designed to demonstrate unique clinical properties and therapeutic effects of directed interferon products which act on HCV and are directed specifically to the hepatocytes. Subsequent trials will follow a traditional 505(b)(2) development program using already-published interferon data as part of its regulatory filing in the US and abroad whenever possible. We anticipate that HDV-INTERFERON will increase available active interferon to the infected hepatocytes and also reduce interferon activity in the periphery unlike the Pegylated Interferon, thereby reducing the adverse effects of the therapy like neuropsychiatric, autoimmune, ischemic, and infectious disorders. If these properties are demonstrated, We believe that HDV-
INTERFERON can constitute a significant therapeutic advancement compared to current Interferon / Pegylated Interferon + Ribavirin products and therefore may merit a priority review. Further, given that Oral HDV-INTERFERON would constitute a first-in-class agent (oral Interferon), and a significant therapeutic advance, a priority review can also be sought.

v. Strategic partnership, merger or acquisition.

To date, more than $20 million has been invested in the development of the HDV platform. We estimate that an additional $7 million will be required to complete the Phase II programs for the two planned products through Phase IIb human studies in India.

We will require additional capital and a strategic partner to produce all forms of HDV-INTERFERON as it advances the program through Phase III and commercialization. We are currently establishing and engaging focused set of partnering discussions with a select group of pharmaceutical companies that have global or regional development and commercialization infrastructures.

We intend to keep the company generally flexible about the structure of a partnership arrangement, but expect a transaction to involve the following economic elements:

- Up-front licensing fee
- Manufacturing and supply agreement
- Sponsorship of costs for clinical trials
- Development milestones
  - Development and Manufacturing relations:
Our development efforts are supported by relationships with both individual consultants and companies. To date, the Company’s primary laboratory research has been conducted in collaboration with SDG, Inc. SDG’s development relationship provides for the necessary formulation, intellectual property and manufacturing support required by Hepasome. In addition, We are in active collaborations with providers of the HDV ingredients.

The production of HDV samples for the clinical trials is done by multiple associates. cGMP manufacturing of HDV and its ingredients is done by Hepasome working with Avanti Polar Lipids, Inc. The HDV final product will be manufactured by Gilead Sciences, Inc. (SDG prepared the Phase IIa HDV blend.). Final product packaging for the HDV for the Phase IIa clinical trials was done by Coldstream Pharmaceuticals.

Our pre-clinical animal studies are led by researchers at both SDG, Inc. and at the Lerner Research Institute at the Cleveland Clinic. The Company’s Phase IIa clinical studies have been directed by Len Rosenberg, the Company’s CEO, in conjunction with iGATE (CRO) along with regulatory consultants as required.
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