Novel antiviral treatment strategies in chronic hepatitis B

A thesis submitted in total fulfilment of the requirements for the degree of Doctor of Medicine

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Abstract

Hepatitis B is a major global health problem with an estimated 2 billion people having been infected with the hepatitis B virus (HBV) and an estimated 350-400 million people suffering chronic infection. Hepatitis B is the 10th leading cause of death worldwide and hepatocellular cancer, a complication of chronic hepatitis B infection, is the 5th most common cancer worldwide. In this thesis I review the virology, epidemiology, natural history and therapy of hepatitis B infection.

The mainstay of medical management of chronic hepatitis B are oral nucleos(t)ide analogues which have the capacity to profoundly suppress viral replication and prevent disease progression. The major shortcoming of nucleos(t)ide therapy is the development of resistance. Therapy for these patients is increasingly difficult in the setting of multi-drug resistance. In this thesis I investigate the use a newer nucleotide, tenofovir disoproxil fumarate, in patients with previous multi-drug (lamivudine and adefovir dipivoxil) resistant hepatitis B. I show tenofovir is safe and effective in such patients, although probably less so than in nucleos(t)ide naïve patients.

Post-liver transplant recurrence of hepatitis B may be severe and life threatening. Standard prophylaxis regimes utilise hepatitis B immune globulin (HBIG) and lamivudine indefinitely post-transplant. In this thesis I investigate the use of combination lamivudine and adefovir dipivoxil prophylaxis, either post-transplant (after a period of HBIG and lamivudine) or pre-transplant (avoiding the use of prolonged HBIG entirely). I show combination adefovir and lamivudine (both pre- and post-transplant) achieves and/or maintains suppression of hepatitis B viral replication and prevents the development of recurrence. This has the benefit of significant cost-savings and is less inconvenient for liver transplant recipients.
Declaration

This is to certify that:

i. the thesis comprises only my original work towards the Doctor of Medicine/Doctor of Medical Science except where indicated in the Preface;

ii. due acknowledgement has been made in the text to all other material used;

iii. the thesis is less than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Signature: ________________________________

Scott John Patterson
Preface

All three studies that comprise this work were conceived by my primary supervisor, Professor Peter Angus and recruited patients from multiple centres in Victoria, New South Wales and New Zealand. Local investigators at these sites managed patients according to study protocol under guidance from myself and Professor Angus. The management of local patients was undertaken by me in conjunction with other hepatologists at my institution. Gilead Sciences (East Melbourne, Victoria) provided adefovir dipivoxil and tenofovir disoproxil fumarate for study. Monitoring during therapy, mandatory ethics reporting, and all work in collating data, analysing results, and interpretation and preparation of the work for publication is my own (with guidance from my supervisors and input from co-investigators in the studies). All statements in this thesis derived from other sources have been acknowledged and properly referenced in the text.
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Gane E, Strasser SI, Patterson SJ, McCaughan GW, Angus PW. A prospective study of the safety and efficacy of lamivudine and adefovir dipivoxil prophylaxis in HBsAg positive liver transplantation candidates. Journal of Gastroenterology & Hepatology 2007;22(s3):A345

Angus PW, Strasser SI, Patterson SJ, McCaughan GW, Gane E. A randomized study to assess the safety and efficacy of adefovir dipivoxil substitution for Hepatitis B Immune Globulin in liver transplantation patients receiving long-term low dose IM HBIG and lamivudine prophylaxis. Hepatology 2007;46:238A.


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Abbreviations

ADV  adeovir dipivoxil
BCP  Basal core promoter
CHB  chronic hepatitis B
cccDNA  covalently closed circular DNA
c/mL  copies per millilitre
DNA  deoxyribonucleic acid
ELISA  enzyme-linked immunoassay
ETV  entecavir
HAART  highly active anti retroviral therapy
HBcAb  hepatitis B core antibody
HBeAb  hepatitis B e antibody
HBeAg  hepatitis B e antigen
HBIG  hepatitis B immune globulin
HBsAb  hepatitis B surface antibody
HBsAg  hepatitis B surface antigen
HBV  hepatitis B virus
HCC  hepatocellular cancer
HDV  hepatitis delta virus
HIV  human immunodeficiency virus
IM  intra-muscular
IU/mL  international units per millilitre
IV  intravenous
kb  kilobase
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAM</td>
<td>lamivudine</td>
</tr>
<tr>
<td>LLOD</td>
<td>lower limit of detection</td>
</tr>
<tr>
<td>LT</td>
<td>liver transplant</td>
</tr>
<tr>
<td>MELD</td>
<td>model for end-stage liver disease</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger RNA</td>
</tr>
<tr>
<td>NK</td>
<td>natural killer</td>
</tr>
<tr>
<td>PBS</td>
<td>pharmaceutical benefits scheme</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>RFLP</td>
<td>restriction fragment length polymorphism</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>TDF</td>
<td>tenofovir disoproxil fumarate</td>
</tr>
<tr>
<td>TNM</td>
<td>tumour node metastasis</td>
</tr>
</tbody>
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Chapter 1. Hepatitis B Virus
1.1 Introduction

Hepatitis B is a major global health problem with an estimated 2 billion people having been infected with the hepatitis B virus (HBV) and an estimated 350-400 million people suffering chronic infection (Maynard 1990). Approximately one quarter of those with chronic infection will die from HBV-related liver disease. The disease results in to 600,000 - 1,156,000 deaths per year and consequently HBV is the 10th leading cause of death worldwide (World Health Organisation 1997; Shepard, Simard et al. 2006). Hepatitis B associated hepatocellular cancer (HCC) alone accounts for >500,000 deaths per year, making it the 3rd most common cause of cancer death worldwide (Parkin, Bray et al. 2005).
1.2 Virology of hepatitis B

1.2.1 The Virus

The hepatitis B virus is a member of the hepadnavirus family. Other members of this family include woodchuck hepatitis virus, duck hepatitis virus and ground squirrel hepatitis virus. All hepadnaviridae are hepatotropic and can cause chronic infection of the liver (Lee 1997; Ganem and Schneider 2001).

The hepatitis B virion (or Dane particle) consists of an outer 42nm diameter spherical lipoprotein envelope and an inner 27nm diameter icosahedral nucleocapsid core enclosing the DNA genome, polymerase and a protein kinase (Dane, Cameron et al. 1970). The outer envelope may extend as a tail on one aspect of the virion due to an overabundance of envelope material. Also present in the serum of HBV infected individuals are non-infectious but immunogenic 22nm diameter spherical particles and rod-like structures comprised purely of excess envelope material. The number of non-infectious sub-viral particles in serum may exceed infectious virions by a factor of 10^3-10^5.

1.2.2 Genome

HBV has a relaxed circular (but not covalently closed), partially double stranded DNA genome. The complete genome is approximately 3200 nucleotides (3.2 kilobases or kb) long. Within an intact HBV particle, the negative sense (non-coding, complementary to viral mRNA) strand is complete and the plus sense strand is incomplete (1.7kb - 2.8 kb in length). After entry into a host cell, HBV DNA is rendered fully double stranded by completion of the plus sense strand and then formed into covalently closed circular DNA (cccDNA) (Beck and Nassal 2007).
The complete HBV genome is comprised of 4 overlapping open reading frames: the envelope (S), core (C), polymerase (P), and X regions (Scaglioni, Melegari et al. 1996). See Figure 1.

![Schematic of HBV Genome & Reading frames](image)

**Figure 1: Schematic of hepatitis B genome**

The envelope reading frame contains three in-phase start codons and a common stop codon which divide it into three overlapping regions of increasing length: pre-S1, pre-S2 and S. These encode for the large (L), medium (M) and small (S or HBsAg) envelope proteins, respectively. The M and S polypeptides are found in all viral and sub-viral particles however the L polypeptide is found predominantly in complete virions. In comparison to the S form, L and M envelope proteins are expressed less abundantly (~5-15% and 1-2% of S expression, respectively).

The core reading frame consists of 2 in-phase start codons that encode for the pre-core and core polypeptides. After translation the pre-core protein is modified into HBeAg.

The largest reading frame encodes for the viral polymerase. It encompasses the envelope region and partially overlaps with both the core and X regions. The X
protein is not required for viral replication however is thought to be a potent promoter of HBV transcription as well as a modulator of numerous host cellular processes.

The HBV genome has two major and several minor RNA transcripts that are produced by cellular (host) RNA polymerase II from the cccDNA template. The two major transcripts are 3.5kb and 2.1kb long and the 3.5kb transcript has two variants. The longer variant commences in the pre-core promoter region and translates into the pre-core protein (and therefore produces HBeAg). The shorter transcript commences in the pre-core region and is translated into the core and polymerase proteins, as well as functioning as a replicative intermediate for HBV DNA genomic reverse transcription (pre-genomic RNA). The second major transcript commences in the pre-S1 region and is translated into the M and S surface proteins. Minor transcripts include a 2.4kb mRNA which encodes for the L surface protein and a smaller RNA which is translated into the X protein.

1.2.3 Replication Cycle

The HBV virion binds to a receptor at the surface of the hepatocyte. Although several candidate receptors have been proposed the precise receptor and mechanism are not known (Schulze, Gripot et al. 2007). It is likely that initial binding is with the L surface protein.

After binding and entry into the host cell, nucleocapsids containing the viral genome travel to the nucleus where the relaxed, partially double stranded DNA is transformed into cccDNA. This cccDNA is the template for the major and minor RNA transcripts, and is an extremely stable component of the replication cycle that is relatively resistant to immune and antiviral effects (Liang 2009). Transcripts are transported to the cytoplasm and then translated into viral proteins: C, pre-C, P, L, M, S and X. Envelope proteins insert themselves into the lipid membrane of the endoplasmic reticulum.

Pre-core protein is modified in the endoplasmic reticulum and secreted as e antigen (HBeAg). The function of HBeAg is incompletely understood: it may be involved in immunologic tolerance however it is not required for replication nor
acute infection in vivo (Milich and Liang 2003). X protein promotes various aspects of HBV replication as well as cellular processes involved in cell proliferation, oncogenesis, and death and is required for viral replication (Murakami 2001).

Pre-genomic RNA (together with the HBV polymerase and a protein kinase) is assembled within nucleocapsid particles. Reverse transcription of pre-genomic RNA into a complete negative sense DNA strand occurs within these core particles. Pre-genomic RNA is later degraded by a nuclease activity of the polymerase protein.

Complete nucleocapsid particles can be assembled into mature virions by the acquisition of an envelope protein coat in the endoplasmic reticulum before being secreted by the golgi apparatus into circulation, or they can re-enter the nucleus to add to the pool of cccDNA and consequently amplify HBV replication. HBV virions may be found in circulation in large quantity: >10^6 copies/mL is not uncommon. Complete virions are relatively enriched for L envelope protein, whereas non-infectious sub-viral particles are comprised predominantly of S envelope protein (and some M).

1.2.4 Antigenicity and Serotypes

All HBV envelope proteins (S, M & L) incorporate the S (or HBsAg) sequence that is highly immunogenic and responsible for potentially protective antibody (anti-HBs or HBsAb).

HBsAg has three distinct epitopes which exhibit antigenicity, one of which is common to all HBV (the ‘a’ determinant, extending from amino acid 124 to 149) and two which each have two possible mutually exclusive sub-determinants (‘d’ or ‘y’ and ‘w’ or ‘r’). This results in 4 possible HBsAg serotypes depending on the combination of determinants: ‘adw’, ‘adr’, ‘ayw’ and ‘ayr’. More complex classification (dividing the ‘w’ and ‘q’ determinants further) is also possible. Due to the common determinant (‘a’) immunity to one serotype of HBV is cross-protective for all others (Szmuness, Stevens et al. 1982). There appears to be no difference in the clinical manifestations of distinct serotypes.
HBsAg serotype distribution is geographic. The ‘d’ determinant is found especially in Northern Europe, the United States, Asia (especially Japan) and Oceania (with the exception of Australian aborigines in whom ‘y’ is more common). The ‘y’ determinant is also more frequent in Africa, India and Mediterranean Europe. In Japan, China and south east Asia, the ‘r’ determinant predominates over the ‘w’, and vice versa elsewhere. Consequently, serotypes ‘adw’, ‘adr’ and ‘ayw’ are found extensively in the appropriate region. Serotype ‘ayr’ is uncommon but found in pockets of the Pacific.

1.2.5 Genotypes

HBV can be divided into 8 genotypes (A-H) on the basis of inter-group genetic sequence variability, which may extend to 8% of the complete genome. Genotyping may be accomplished by analysing only a portion of the genome (typically the S sequence) by several techniques: direct sequencing, restriction fragment length polymorphism (RFLP), line-probe assay, enzyme linked immunoassay (ELISA) or PCR with genotype specific primers. As with HBsAg serotypes, more complex genotypic classification is possible (sub-grouping genotypes A, B and C).

Genotypes A-D, which account for most infection, have distinct geographic distribution. Genotype A is common in Northern Europe, North America and Africa. Genotype B and C are common in Asia and genotype D is common in Mediterranean Europe, the Middle East and India.

Genotypes have been shown to influence clinical outcome of infection and response to therapy. This has been most clearly demonstrated with genotypes B and C in studies from Asia. When compared to genotype B, genotype C appears clinically more aggressive: there is generally HBeAg positive disease, more active inflammation, increased progression to cirrhosis and HCC, and an inferior response to therapy (Sumi, Yokosuka et al. 2003; Chu and Liaw 2005). There are few studies regarding clinical differences between the other genotypes, however pre-core mutant HBV (see 1.2.6) seems more common in genotypes D and B (3%,
46%, 24%, and 74% in genotypes A, B, C and D, respectively) (Chu, Keeffe et al. 2003).

1.2.6 HBV Variants

Errors during replication can lead to the creation of hepatitis B variants or mutants. DNA viruses are generally less susceptible to errors of replication than RNA viruses due to the presence of a proofreading function. Per replication cycle, DNA viruses might sustain approximately 1 mutation per $10^8$ nucleotides whereas in RNA viruses there may be 1 mutation per $10^3$-$10^5$ nucleotides. The requirement for reverse transcription of an RNA transcript during HBV replication accounts for a higher than expected mutation rate of approximately 1 mutation per $10^5$-$10^6$ nucleotides (Girones and Miller 1989).

Selection pressures such as the host immune response, antiviral therapy, and competition based on replication fitness leads to the development of HBV variants (or mutants) that confer an advantage to the virus. Most variants are not viable, in part due to the overlapping reading frames of HBV. Many variants however, may co-exist within the one patient and are termed quasi-species. Common HBV variants are categorised on the basis of their location within the genome.

1.2.6.1 Pre-core variants

A common HBV variant is the point mutation substitution of an adenine for a guanine nucleotide at position 1896 (G1896A). This has been found in 27% of chronic hepatitis B patients in the United States (9% of HBeAg positive patients and 38% of HBeAg negative patients) (Chu and Liaw 2005), and creates a premature stop codon in the pre-core reading frame that aborts translation of pre-core protein (HBeAg). This stop codon has no effect on core protein synthesis since it is upstream of the core start codon and core protein is translated from a separate RNA transcript. Although pre-core variant HBV is more common in patients with HBeAg negative chronic hepatitis B, it has also been found in HBeAg positive chronic hepatitis B. As discussed, pre-core variant hepatitis B is more common in genotype D and B infection (Chu, Keeffe et al. 2003).
A number of other pre-core variants have been described including point mutations in the pre-core start codon and frame-shift mutations (insertions or deletions).

Selection of pre-core variant HBV is probably driven by immunological selection pressure targeting HBeAg. This is supported by the observation that HBeAg positive patients with pre-core variant HBV are more likely to experience HBeAg seroconversion (Lok, Akarca et al. 1995). Although pre-core protein has been shown to have a negative effect on HBV replication in vitro, pre-core variant HBeAg negative chronic hepatitis B disease is usually associated with relatively low levels of viral replication (Scaglioni, Melegari et al. 1997).

1.2.6.2 Basal core promoter variant

The most common HBV variant is a dual mutation in the basal core promoter (BCP) region: A1762T and G1764A, which has been described in 44% of chronic hepatitis B patients in the United States (Chu and Liaw 2005). This variant decreases the transcription of pre-core mRNA and therefore HBeAg production. In cross-sectional studies this dual BCP variant HBV has been associated (independent of HBV genotype) with increased prevalence of cirrhosis and HCC (Yuen, Tanaka et al. 2004). Several other mutations have been found in association with this dual BCP mutation. Other deletions have also been described within the BCP region however these are usually associated with very low levels of HBV replication (Laskus, Rakela et al. 1994).

1.2.6.3 Pre-S/S variant

The ‘a’ determinant region of HBsAg is the major epitope involved in humoral immunity. The most common HBsAg mutation is G145R, however a variety of mutations in this region may give rise to vaccine-escape, or the development of chronic hepatitis B despite an adequate prior HBsAb response to vaccination. Not surprisingly, the prevalence of S mutations has been observed to increase following the introduction of universal vaccination. For example the prevalence of S mutations in HBsAg positive Taiwanese children increased from 7.8% in 1984 to 25% in 1994 (Hsu, Chang et al. 2004). The carriage of HBV has decreased
substantially (9.8% to 0.7%) over the same period, suggesting that the overall efficacy vaccination is not significantly compromised.

S variant HBV is also observed in the context of immuno-prophylaxis with hepatitis B immune globulin (HBIG) following liver transplantation (Ghany, Ayola et al. 1998). Pre-S/S variant HBV may also explain the phenomenon of occult HBV, or detectable HBV virus in the absence of detectable HBsAg (Kato, Hasegawa et al. 1996). This has an impact on blood product screening, organ donation and establishing the aetiology of cryptogenic hepatitis.

1.2.6.4 Polymerase mutations

Although they can arise spontaneously, mutations in the polymerase region are usually detected in the context of exposure to oral antiviral therapy (see 1.6.3).

1.2.6.5 X gene variants

The X gene has important roles in promoting and regulating both viral and host genes and may be important in hepatic carcinogenesis. The two most common X gene mutations correspond (as a consequence of overlapping reading frames) to the dual BCP mutation A1762T and G1764A. The relative importance of the disturbance of X or core protein synthesis as a result of these mutations is unclear (Baptista, Kramvis et al. 1999).
1.3 Epidemiology of hepatitis B

1.3.1 Distribution, Incidence & Prevalence

Hepatitis B has a worldwide distribution. More than a third of the global population has been infected with HBV, and about 5% suffer from chronic infection. Prevalence of hepatitis B varies between <1% (typically wealthy Western nations) to >10% (in parts of China, Southeast Asia and Northern Africa) (Dienstag 2008). See Figure 2.

The prevalence of HBV carriage is closely related to the incidence and the age of primary infection, as infection in adulthood rarely progresses to chronic infection. In HBV-prevalent areas such as Alaska, Taiwan and Indonesia, large-scale infant vaccination campaigns have been demonstrated to lead to a major fall in prevalence.

The incidence of hepatitis B is not seasonal. Epidemics of symptomatic hepatitis B are rare, and usually related to systematic use of contaminated or unsterilized medical equipment.

1.3.2 Transmission

There are four recognized modes of HBV transmission: perinatal, horizontal, sexual and parenteral. Although HBsAg has been found in all body secretions and excretions, only blood, vaginal and menstrual fluids, and semen have been shown to be infectious. There is no documented evidence for airborne or faecal-oral infection, nor is HBV transmitted by insects or other vectors.

Perinatal transmission occurs from an infected mother to her child at birth and this is the dominant mode of transmission in areas of high prevalence. The mechanism is thought to be neonatal mucous membrane exposure to maternal blood or body fluids. Transmission is more likely if the mother has a high viral load or is HBeAg positive (which may enhance tolerance and thereby promote
**Figure 2: Worldwide variation in prevalence of HBsAg**

High >8%, Intermediate 2-8%, Low <2%. Illustration adapted from Wikipedia with permission, verified to be accurate (data source: Dienstag et al, 2008).

chronic infection in addition to being associated with high viral load) (Milich and Liang 2003).

Horizontal transmission occurs by close household contact with an infected individual (without sexual contact). The likely mechanism is inadvertent or unrecognised exposure to blood either directly (close play) or by means of contaminated object (baby bottle, toy, cutlery, toothbrush, etc.). This mode of exposure is common in infants within highly prevalent areas, and particularly in Africa. In addition, traditional rituals (in particular scarification) are a potential mechanism of transmission in Africa.

Parenteral transmission may result from recognised blood or other infectious fluid exposure. This may be medical by means of unscreened blood or blood products, haemodialysis, inadequately sterile vaccination practices; occupational by needle-stick injury or sharp exposure; recreational by sharing unsterilized intravenous equipment; or by other means including tattooing, body piercing, acupuncture or contact sport. The risk of transmission of HBV via a needle-stick
injury ranges from 1-30% depending on the HBeAg status of the source (Werner and Grady 1982; Centers for Disease Control and Prevention 2001).

It is recognised that mode of exposure is impossible to determine with certainty in up to 35% if patients.

1.3.3 Prevention of HBV Transmission

Education may prevent behaviours associated with a risk of exposure to HBV. Examples of such education initiatives include sex education as to the risk of sexual transmission (of HBV or other sexually transmitted diseases), or safe injecting practices for intravenous drug users. Groups at increased risk of contracting hepatitis B infection are listed in Table 1.

<table>
<thead>
<tr>
<th>Infants of HBsAg positive mothers</th>
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<tr>
<td>Young children in close contact with others in endemic areas</td>
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<tr>
<td>Sexual/household contacts of infected individuals</td>
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<td>Health care workers</td>
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<td>Haemodialysis patients</td>
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<td>Intravenous drug users</td>
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<td>Sexually active persons</td>
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<tr>
<td>Travellers to areas endemic for HBV</td>
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<tr>
<td>Patrons or staff of unsterile tattooing or body piercing services</td>
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**Table 1: Groups at risk of acquiring HBV**

Mandatory screening of blood products has dramatically reduced the possibility of acquiring hepatitis B infection via transfusion. In Australia, blood donors are screened by HBsAg testing and a questionnaire regarding high-risk behaviours and relevant past history. Patients in the incubation phase of infection may not have detectable levels of HBsAg and therefore the objective of a no-risk blood supply is not achievable. The risk of acquiring HBV from a single blood donation
in Victoria, Australia has been estimated as 1 in 155,000 donations, or 0.0006%. The risks of hepatitis C or HIV infection are 1 in 234,000 donations (0.00043%) and 1 in 1.27 million donations (0.000079%) respectively.

1.3.3.1 HBIG Immunoprophylaxis

The first successful pre-exposure prophylaxis of hepatitis B infection was achieved with Hepatitis B Immune Globulin (HBIG), an immunoglobulin preparation from blood donors with high titres of antibody to HBsAg (i.e., HBsAb or anti-HBs). This passive immunoprophylaxis provides an immediate but limited duration (3-6 months) of protection from acquiring HBV. This product is expensive and of limited availability, and the limited duration of efficacy precludes its widespread use as pre-exposure prophylaxis.

In addition to providing protection prior to exposure, HBIG may also be effective if administered within 48 hours following exposure (i.e., post-exposure prophylaxis). HBIG reduces HBV transmission in adults by approximately 75% and reduces the incidence of maternal-neonatal transmission by approximately 70% (Grady, Lee et al. 1978; Beasley, Hwang et al. 1983). HBIG therapy has few side effects and the process by which HBIG is manufactured inactivates HIV.

HBIG has also been used extensively in long term prevention of HBV reactivation following liver transplantation for hepatitis B related disease (see section 1.7), particularly in combination with oral anti-HBV agents.

1.3.3.2 HBV Vaccination

Active vaccination against hepatitis B has been available for more than 20 years and provides durable pre-exposure prevention of HBV transmission. As man is the natural reservoir for HBV, as well as the only vector for transmission, HBV has the potential to be eradicated by vaccination. The majority of those with chronic hepatitis B disease are infected during infancy, therefore national vaccination programs in HBV prevalent areas are targeted at neonates. A reduction in the prevalence of HBV has been achieved in several highly endemic areas following the introduction of infant immunization campaigns. For example in Taiwan, a universal infant vaccination program instituted in the mid 1980s has been 78-
87% effective in decreasing the seroprevalence of HBsAg (Chien, Jan et al. 2006). It has been estimated that 85-90% of the deaths due to HBV disease (~half a million deaths per year) are vaccine preventable.

The first HBV vaccines to become available were plasma-derived products from HBsAg positive donors. This HBsAg was purified, inactivated (by formalin and/or heat), and adsorbed to aluminium. The resultant particles were free from nucleic acids and therefore non-infectious.

More recently recombinant DNA technology has allowed isolation and expression of the S gene in cultured yeast or mammalian cell lines. The expressed HBsAg protein is assembled onto antigenic particles. This method of vaccine production removes the constraints of supply that are inherent in the use of a plasma-derived product. Various recombinant HBV vaccines differ in their glycosylation of HBsAg and in the degree to which other S gene related products (pre-S1 or pre-S2) are incorporated.

Regardless of the sources of the HBsAg the epitope of the HBsAg protein that elicits the most important protective immune response is the ‘a’ determinant. Vaccination is delivered by intramuscular injection and for optimum immunogenicity a course of HBV vaccination comprises three injections 1 and 6 months after the first. An adequate initial response to vaccination is the achievement of a HBsAb titre of >10IU/L. If this initial response is achieved, protection is durable even if HBsAb levels fall due to the development of a rapid anamnestic response in most vaccine recipients. The duration of immunity is not known, but is thought to be at least 15 years and booster vaccinations are not routinely recommended.

Factors that may diminish the efficacy of HBV vaccination include recipient age (>40), obesity, haemodialysis, chronic livers disease, HIV infection or other immunosuppression, smoking, subcutaneous administration, improper storage and an accelerated schedule of vaccination. Post–vaccination testing to document serologic response is only recommended in infants of HBsAg positive mothers, health care workers or others with occupational HBV exposure,
immunosuppressed patients (including haemodialysis or HIV positive patients) and sexual partners of those with chronic hepatitis B infection. Vaccination of individuals infected with chronic hepatitis B is safe but ineffective in inducing measureable a HBsAb titre.

Post-exposure prophylaxis incorporating HBIG and vaccination is thought to decrease the risk of HBV transmission to <5% (Mitsui, Iwano et al. 1989). HBV vaccination has therefore been incorporated into post-exposure protocols generally.
1.4 Pathogenesis of hepatitis B

In most circumstances HBV appears to be non-cytopathic and hepatocyte injury is related to host immune responses to the virus.

Several clinical observations support the predominant role of immune-mediated liver injury in the pathogenesis of hepatitis B disease. Firstly, high levels of HBV seen in the immune tolerant phase of infection (see 1.5.2.1) are associated with little or no inflammation or hepatocyte injury (McMahon 2009). Secondly, flares of hepatitis B (including fulminant hepatitis and massive hepatocyte injury) are often associated with low or undetectable levels of HBV as a consequence of successful underlying immune clearance. Similarly, other potential immune clearance events (interferon therapy or HBeAg seroconversion) are frequently associated with flares in biochemical and histological activity. Thirdly, immune suppression is often associated with increased viraemia although clinical hepatitis may not occur until the immune suppression abates. An example of this is "immune restoration disease" which may be seen following successful therapy against human immunodeficiency virus (HIV) in HBV/HIV co-infected patients (Crane, Oliver et al. 2009). An untreated, immuno-compromised patient with HIV/HBV co-infection often has high levels of HBV replication without evidence of liver injury. With restititution of immune competency by highly active anti-retroviral therapy (HAART), a flare of hepatitis and fall in HBV viral load are often observed (den Brinker, Wit et al. 2000). A further example of "immune restoration disease" is hepatitis B reactivation following cessation of immunosuppressive chemotherapy for malignancy.

Infected hepatocytes expressing HBV epitopes (in the context of major histocompatibility complex type II molecules and necessary co-stimulation) undergo lysis by specific host T-cells or natural killer (NK) cells. Indeed, acute hepatitis is associated with a strong, polyclonal, multi-specific cytotoxic T
lymphocyte response to the viral envelope, nucleocapsid and polymerase proteins that persists for decades after clinical recovery (Rehemann, Lau et al. 1996).

Rarely, HBV may be directly cytopathic to hepatocytes when there are very high levels of viral replication. For example, following liver transplantation, the lesion of fibrosing cholestatic hepatitis, which can lead to rapidly progressive graft failure, is associated with extremely high levels of viral replication but little evidence of immune mediated injury (Davies, Portmann et al. 1991).
1.5 Clinical manifestations of hepatitis B

1.5.1 Acute hepatitis B

The incubation period from acute HBV exposure to symptomatic disease ranges from 4-26 weeks (average 10 weeks). Hepatitis B surface antigen (HBsAg) becomes detectable in the blood during the incubation phase of infection. Approximately 30% of acute hepatitis B is subclinical or anicteric. Symptomatic hepatitis may last up to 3 months and typically comprises fever, jaundice, malaise, anorexia, nausea, abdominal pain, myalgia, arthralgia, rash, and dark urine.

The majority of adults who acquire hepatitis B will mount a strong immune response and clear the virus. The rate of clearance of adult-acquired acute HBV has been estimated as >95% (one Greek study estimated 99.8%) (Tassopoulos, Papaevangelou et al. 1987). In patients who successfully clear the virus, HBsAg titres peak and fall before the appearance of hepatitis B surface antibody (HBsAb) that is protective against future infection. These individuals will usually also express hepatitis B core antibody (HbcAb) indefinitely, and this distinguishes them from a post-vaccination state (HBsAb positive but HbcAb negative).

Following clearance, patients have traditionally been thought to harbour no detectable HBV, however using sensitive polymerase chain reaction (PCR) -based assays HBV may be detected in the liver (and less frequently serum). This explains the possibility of reactivation of acute HBV in HBsAg negative, HBsAb and HbcAb positive immuno-suppressed patients as well as the potential for transmission of HBV via a liver transplant allograft from an HbcAb positive organ donor (Wachs, Amend et al. 1995; Ahn, Park et al. 2005).

Acute HBV infection may trigger massive hepatic necrosis and this corresponds to a fulminant presentation and incipient liver failure in 0.1%-0.5% of cases. Fulminant HBV is potentially fatal without liver transplantation. Viraemia is often absent and this reflects the overwhelming immune response to infection (the
mechanism for the massive hepatic necrosis). Therefore, despite the severity of the illness, fulminant hepatitis B paradoxically augurs well for HBV clearance.

### 1.5.2 Chronic hepatitis B

Failure to clear HBV following acute infection results in chronic hepatitis B infection. This is defined as the presence of HBsAg for >6 months following acute infection. Most adults acutely infected with hepatitis B develop a vigorous immune response that results in clearance of HBsAg. However, the vast majority of neonates and young children fail to mount an effective immune response to the virus. As a result, acute infection is usually asymptomatic and commonly followed by the establishment of chronic, lifelong infection which may not produce symptoms until the development of cirrhosis and its complications (see section 1.5.3).

It is now recognised that the natural history of chronic hepatitis B disease may involve a number of different phases that influence both the rate of disease progression and the response to treatment (McMahon 2009). Characteristics of these phases are illustrated in Figure 3.

#### 1.5.2.1 Immune tolerant phase

Most neonates and young infants initially enter the immune tolerant phase of chronic hepatitis B infection. In this phase, despite high levels of viral replication, there is little immune response and therefore liver enzymes are usually not elevated and histological activity of hepatitis is mild or absent. As a consequence, progressive liver injury and fibrosis does not occur. Hepatitis B e antigen is present.

The immune tolerant phase can last well into the third decade of life. Most adults who acquire chronic HBV usually do not enter an immune tolerant phase and move directly into attempted immune clearance.
**Figure 3: Characteristics of the phases of chronic hepatitis**

### 1.5.2.2 Immune clearance (HBeAg positive chronic active hepatitis) phase

The immune clearance phase is characterised by fluctuating hepatitis B viral loads, persistence of HBeAg and episodes of immune mediated liver injury heralded by elevations in liver enzymes. There is active hepatic inflammation and this can lead to progressive liver fibrosis. Patients with hepatitis B acquired neonatally or as young infants commonly enter this phase in young adulthood.
Patients may remain in this phase indefinitely (usually acquiring progressive liver damage) or the may exit the phase in one of three ways. The most common (~75%) is to achieve HBeAg seroconversion in which HBeAg is lost and HBeAb appears and a state of immune control is achieved (McMahon, Holck et al. 2001). Spontaneous progression to the immune control phase has been estimated to occur in 5-10% of patients annually (Fattovich, Rugge et al. 1986). Hepatitis B e antigen may be lost transiently; approximately 20% of patients who appear to exit the immune clearance phase promptly revert back to it (McMahon, Holck et al. 2001).

1.5.2.3 Immune control (inactive carrier) phase

The immune control phase is characterised by low hepatitis B viral loads, serum markers of hepatitis within the normal range, and absent histological inflammation. Consequently progressive liver injury does not occur, although liver fibrosis may have accumulated from the immune clearance phase.

The immune control phase represents successful suppression of the disease by the host immune system. This phase is regarded as an important endpoint of therapy for patients who begin treatment with HBeAg positive disease.

Immune control is generally durable, however it may end either spontaneously or in the context of immunosuppression (Lok, Liang et al. 1991).

1.5.2.4 Immune escape (HBeAg negative chronic active hepatitis) phase

The immune escape phase is characterized by reactivation of viral replication following a period of immune control. Viral loads are moderate to high and there is evidence of reactivation of liver injury. There may be flares of increased activity or inflammation may be relatively constant. Progressive liver injury can lead to advanced fibrosis and cirrhosis. Few patients undergo spontaneous remission of this phase (<15% overall) (Hadziyannis and Vassilopoulos 2001).

The loss of HBeAg in this phase occurs via the development of pre-core mutation or basal core promoter mutation and allows for evasion of the host immune
response targeting e antigen. The pre-core mutation is particularly common in genotype D patients from the Mediterranean (>80%) whereas both pre-core mutation and basal core promoter mutation are present in ~40% of Asian patients with HBeAg negative chronic hepatitis (Chan, Leung et al. 2000; Jardi, Rodriguez et al. 2004).

1.5.2.5 Clearance of chronic infection

Clearance of chronic hepatitis B is signified by loss of hepatitis B surface antigen and the appearance of hepatitis B surface antibody. This occurs spontaneously at a rate of approximately 0.5-2% per year usually at the conclusion of the immune clearance phase. It is very rare for this to occur in patients who have entered the immune escape phase (Liaw, Sheen et al. 1991). Patients who have achieved HBsAg clearance remain at increased relative risk (compared to those never exposed to hepatitis B) of the development of hepatocellular carcinoma and reactivation is possible usually in the setting of profound immunosuppression (Ahn, Park et al. 2005).

1.5.3 Progression to cirrhosis

The prognosis of chronic hepatitis B is difficult to predict with certainty in the individual patient. Overall approximately 2-3% of patients per year will develop cirrhosis and the estimated 5-year rate of progression from cirrhosis to decompensated chronic liver disease is 20% (Di Marco, Lo Iacono et al. 1999). The 5 year survival of cirrhotic patients has been estimated at 85% however decompensated cirrhosis is associated with only a 14-35% survival at 5 years (Fattovich, Bortolotti et al. 2008). It has been estimated that worldwide 40-50% of men and 15% of women with chronic hepatitis B will die from complications of their liver disease (Beasley, Lin et al. 1982).

Apart from male sex, there are several factors that have been associated with progression to cirrhosis. These include increasing age, high viral load, presence of hepatitis B e antigen, and elevated serum markers of hepatitis (Yu, Hsu et al. 1997; Lloëje, Yang et al. 2006).
Although compensated cirrhosis may be asymptomatic there may be clinical stigmata of chronic liver disease such as spider naevi, palmar erythema, fingernail clubbing, (and gynaecomastia in males). Alternatively the diagnosis may be suspected on the basis of imaging findings or blood testing (hypoalbuminaemia, thrombocytopenia). Often, however, cirrhosis may go unrecognized until the development of decompensated cirrhosis. These complications include: ascites, spontaneous bacterial peritonitis, variceal haemorrhage, hepatic encephalopathy, hepato-renal syndrome, hepato-pulmonary syndrome.

1.5.4 Hepatocellular Cancer

Liver cancer is the sixth most common cancer worldwide with 626,000 new cases diagnosed per year and the vast majority of these cases are hepatocellular cancer. Due to a high mortality rate (598,000 deaths per year) it is the 3rd most common cause of cancer death (Parkin, Bray et al. 2005). The majority (>80%) of hepatocellular cancer occurs in developing countries and is more common in men than women (male:female ratio 2.4:1).

It has been long recognised that individuals with chronic hepatitis B infection have a markedly increased relative risk of developing hepatocellular cancer. In a large prospective study of 22,000 Taiwanese males, HBsAg seropositivity was associated with a 63-fold risk of developing hepatocellular cancer when compared to HBsAg negative controls (Beasley, Hwang et al. 1981; Beasley, Lin et al. 1982). In this study HCC accounted for 54% of the deaths observed in the HBsAg positive cohort (Beasley 1988). Other studies from hepatitis B endemic areas have confirmed this significant increase in relative risk. Contrastingly a study from Northern Italy with 29 years of follow-up observed no difference in the incidence of HCC amongst HBsAg positive prospective blood donors when compared to HBsAg negative controls (Manno, Camma et al. 2004). This wide disparity in HCC risk in different HBsAg positive patient populations is likely to reflect the fact that in Asia most patients have neonatally acquired HBV and high rates of cirrhosis whilst in the Northern Italian population HBV is often acquired later in life.
Other liver diseases associated with hepatocellular cancer include cirrhosis in particular due to chronic hepatitis C infection but also due to alcohol abuse, non-alcoholic fatty liver disease, haemochromatosis or alpha-1 anti-trypsin deficiency (Di Bisceglie 2009; McMahon 2009). However, in countries where HBV is endemic (especially within Asia and Africa) it is by far the most important risk factor for HCC, estimated to be the aetiology in over 60% of cases, whereas in Western societies HBV contributes to 20% of the burden of HCC disease (Bosch, Ribes et al. 2004).

Contrary to most other chronic liver diseases, 30-50% of HCC complicating chronic hepatitis B occurs in the absence of cirrhosis (Bosch, Ribes et al. 2005). Several studies have demonstrated that the increased risk may be at least partly related to viral replication (Ohkubo, Kato et al. 2002; Yang, Lu et al. 2002; Tang, Kruger et al. 2004; Chen, Yang et al. 2006). The largest of these studies (a prospective cohort study of 3653 Taiwanese patients), in addition to reaffirming that baseline viral load $>10^5$ copies/mL was associated with the later development of HCC also demonstrated that this association was independent of ALT, presence of HBeAg or baseline cirrhosis (Chen, Yang et al. 2006). It has also been shown that in Asian patients genotype C chronic hepatitis B has a greater predisposition to the development of HCC than genotype B infection (Orito, Ichida et al. 2001).

The mechanism by which HBV contributes to the development of HCC is incompletely understood. Various mechanisms including incorporation of viral DNA into critical regions of the host genome; transcriptional activation of cellular growth genes by viral protein products (particularly X protein, also the truncated surface protein); and inhibition of natural tumour suppressor pathways have been implicated (Blum and Moradpour 2002).

The prognosis of HCC is poor. This may be in part because it is often diagnosed late. However, survival is relatively poor even when the disease is diagnosed early. Stage I in the Tumour Node Metastasis (TNM) classification is associated with only a 55% 5-year survival (Vauthey, Lauwers et al. 2002). Screening at-risk patients is recommended by consensus guidelines on the basis of a survival
benefit (McMahon, Bulkow et al. 2000; Bruix and Sherman 2005). This includes HBsAg positive patients over the age of 40 or those with cirrhosis, active liver disease with high viral load, or with a family history of HCC. Screening protocols generally include 6-monthly alpha-fetoprotein quantification and 6-12 monthly liver ultrasonography.
1.6 Therapy of hepatitis B

The general management of chronic hepatitis B encompasses many aspects. These include screening at-risk patients, counselling with regard to the to prevention of transmission, vaccination of close contacts, lifestyle modifications (particularly avoiding consumption of alcohol in excess), screening for common co-infection (hepatitis C and possible hepatitis delta and/or human immunodeficiency virus), vaccination against hepatitis A, and monitoring of disease activity and HCC screening (Lok and McMahon 2007). In addition many patients will eventually develop active liver disease that warrants antiviral therapy. When considering antiviral treatment it is useful to identify into which of the previously described phases of disease the patient currently falls. Viral load, ALT level and even HBeAg status may vary with serial measurements and it is often necessary to monitor patients over many months before a reliable pattern is established.

In general therapy is not recommended for patients in the immune tolerant or immune control phases of infection. Patients with persistently elevated ALT levels or patients > 40 years of age with persistent viral replication despite normal ALT levels should be considered for liver biopsy and treatment. All patients with decompensated cirrhosis, regardless of the aetiology or phase of chronic HBV, should have nucleos(t)ide-based antiviral therapy because of the risk of death with upon flare or reactivation.

Liver biopsy remains the most accurate method to determine the degree of liver injury (activity of inflammation and stage of fibrosis) and to exclude other causes of liver disease. In Australia, a liver biopsy demonstrating active hepatitis is required (unless contraindicated) prior to subsidised treatment under the Pharmaceutical Benefits Scheme. However, liver biopsy is invasive, has an attendant risk of serious complication, and is subject to sampling error. Liver biopsy may be particularly useful in patients with less significant biochemical
hepatitis (ALT only slightly elevated or high-normal) or to correctly identify patients with possible early cirrhosis.

A detailed discussion of the available HBV treatment options follows. Regardless of the agent used, patient compliance to treatment is of paramount importance for maximum efficacy. Particularly during chronic therapy with oral anti-HBV agents, non-compliance to therapy is a frequent cause of sub-optimal response and increases the risk of drug resistance (Lok and McMahon 2007; Ghany and Doo 2009).

1.6.1 Treatment endpoints

The immediate goal of anti-HBV therapy is to suppress viral replication and allow for remission of hepatitis. The ultimate aim of therapy is to prevent progression of liver injury and thereby the development of cirrhosis and its complications. There is evidence to suggest that suppression of viraemia might also be associated with a decrease in the risk of the development of HCC (Liaw, Sung et al. 2004; Chen, Yang et al. 2006).

Successful response to anti-HBV therapy is assessed by its effects on liver biochemistry, HBV viral loads and liver histology. These responses have been formally defined for the purposes of clinical studies (Lok and McMahon 2007; Zoulim and Locarnini 2009). A biochemical response is defined as a reduction in ALT to the normal range whereas a histological response requires a decrease in the histological activity index by 2 points and no progression in fibrosis. A virological response is defined as a reduction in HBV viral load to undetectable levels (current standard is by a PCR-based assay) and a loss of HBeAg in patients initially HBeAg positive. Primary non-response (failure of at least a partial virological response) is defined as $< 1 \log_{10} \text{IU/mL}$ fall in HBV viral load despite 12 weeks of therapy (it is variously classified as a $<2 \log_{10} \text{IU/mL}$ fall in HBV viral load despite 24 weeks of therapy). Finally, a complete response occurs rarely and is heralded by a loss of HBsAg in addition to fulfilling the biochemical and virological response criteria. Response may assessed whilst on-treatment, at end-of-treatment, or at certain time points following treatment (termed a sustained
response). Virological relapse is defined as an increase in viral load by $>1 \log_{10}$ IU/mL following therapy.

For patients who are initially HBeAg positive, HBeAg to HBeAb seroconversion may indicate a durable transition to the immune control phase. HBeAg seroconversion is therefore a surrogate marker of sustained suppression of viral replication that may allow for cessation of therapy.

### 1.6.2 Interferon-based therapy

Interferons are a family of cytokines which have a range of actions has several actions: immunomodulatory, anti-proliferative and antiviral. Interferon-alpha was the first agent to be successfully used in the treatment of HBV (Lok, Weller et al. 1984). The antiviral effects of interferon-alpha include stimulation of cell-mediated immune mechanisms targeting HBV infected cells, and increased intracellular breakdown of viral RNA (Perrillo 2009). Recently interferon-alpha has been pegylated (combined with a polyethylene glycol molecule) to allow less frequent administration.

Interferon-alpha based anti-viral therapy is complicated by frequent, potentially serous, side effects. Common side effects include “flu-like” symptoms of fever, chills, myalgias, arthralgias, headaches and fatigue; alopecia; weight loss; and asymptomatic thrombocytopenia or leukopaenia. More significant side effects include neutropaenia, thrombocytopenia, retinopathy, thyroid dysfunction, and potentially severe exacerbations of depression. These side effects lead 31%-47% of patients to require dose reductions and 6%-9% discontinue therapy prematurely (Perrillo 2009).

Interferon therapy is associated in 30-40% of cases with a “flare” of hepatitis - an increase in the activity of hepatitis manifested by increased ALT. This phenomenon is a consequence of increased immune clearance and a favourable marker of potential response. Flares are usually sub-clinical but in the setting of decompensated cirrhosis can be severe and potentially life threatening. Decompensated cirrhosis is therefore a contraindication to interferon therapy.
1.6.2.1 Standard Interferon

Standard interferon-alpha is administered by subcutaneous injection, usually either 5 million units (MU) daily or 10 MU thrice weekly (Lok and McMahon 2007). Interferon-alpha is available in two preparations, interferon alpha-2a and interferon-alpha-2b, the major difference being in their pharmacokinetic properties.

A meta-analysis of 15 controlled studies involving 837 HBeAg positive patients found significantly better responses in those randomized to standard interferon rather than placebo. Standard interferon was associated with loss of HBeAg in 34% (12% with placebo), reduction of HBV viral load to undetectable levels by a hybridisation assay in 37% (17% with placebo), and loss of HBsAg in 7.8% (1.8% with placebo) (Wong, Cheung et al. 1993). Seroconversion to HBeAb and normalisation of ALT levels was also significantly more frequently observed with interferon. Treatment in these studies was for 3-6 months and responses were assessed at 6-12 months following end of treatment.

Standard interferon-alpha is less effective in patients with HBeAg negative chronic hepatitis. Although end-of treatment virological responses are observed in 38-90% of patients, >50% of patients relapse following cessation of therapy (Papatheodoridis, Manesis et al. 2001; Lok and McMahon 2007). In one study 85.7% of patients with HBeAg –ve disease relapsed following an end-of treatment virological response compared to 19.4% of patients with wild-type HBeAg positive virus (Brunetto, Giarin et al. 1993). Longer courses of standard interferon-alpha have been used in HBeAg negative patients with improved results. With 24 months of therapy, approximately one third of patients have a sustained virological response when assessed by hybridisation assay (limit of sensitivity ~10-100,000 copies/mL) (Lampertico, Del Ninno et al. 1997).

1.6.2.2 Pegylated Interferon

Pegylated interferon-alpha has largely replaced standard interferon-alpha in the treatment of hepatitis B. It is also administered by subcutaneous injection however the pegylated form has the advantage of a longer pharmacological half-
life due to decreased clearance and this favourable pharmacokinetics allows for once weekly dosing.

The largest studies of pegylated interferon therapy for hepatitis B included 814 HBeAg positive and 537 HBeAg negative patients and compared 12 months of therapy with pegylated interferon alone, lamivudine alone, and combination pegylated interferon and lamivudine (Marcellin, Lau et al. 2004; Lau, Piratvisuth et al. 2005). In the HBeAg positive study, although end-of-treatment viral suppression was greatest in the combination pegylated interferon and lamivudine group, at 6 months following therapy significantly more patients in the interferon groups had a virological response when compared to the lamivudine group (33% vs. 22% using a threshold 100,000 copies/mL and 14% vs. 5% using a threshold of 400 copies/mL, p<0.001). HBeAg seroconversion at 6 months post-treatment was also significantly more common in the group receiving pegylated interferon (32% vs. 19%, p<0.001), as was a complete response (a combined biochemical, serological and <400 copies/mL virological response: 23% vs. 10%, p<0.001). In the HBeAg negative study, sustained viral suppression 6 months following therapy was also significantly greater with pegylated interferon compared to lamivudine (43% vs. 29% using a threshold of 20,000 copies/mL and 19% vs. 12% using a threshold of 400 copies/mL, p=0.003), as was a complete response (15% vs. 6%, p=0.007). In both these studies the addition of lamivudine to pegylated interferon had no significant effect on 6-month post-treatment responses. Interestingly, these studies found a 3-7% rate of HBsAg clearance in patients receiving pegylated interferon (wither alone or in combination) but not lamivudine alone. HBsAg clearance also been observed in long-term follow-up of patients successfully treated with standard interferon. This is an endpoint rarely reached with nucleos(t)ide therapy (Perrillo 2009).

There are a number of predictors of successful response to interferon-based therapy of which the most important are a high baseline ALT level and low pre-treatment viral load (Lok and McMahon 2007). Other predictors include histological activity index, hepatitis B genotype and quantitative HBeAg level (Janssen, van Zonneveld et al. 2005; Fried, Piratvisuth et al. 2008).
1.6.3 Nucleoside and nucleotide analogues

The nucleoside and nucleotide analogues inhibit the HBV polymerase/reverse transcriptase enzyme that is critically required in the replication cycle of HBV. HIV also requires reverse transcription to complete its viral replication cycle, and a number of nucleos(t)ide anti-HBV agents were initially used in the treatment of HIV/AIDS.

As a class, the nucleos(t)ide analogues have revolutionized the management of chronic hepatitis B. These agents are administered orally, produce profound suppression of viral replication and are generally safe and well tolerated even with long-term therapy (Fontana 2009). Successful nucleos(t)ide therapy prevents progression of fibrosis and may allow for the regression of fibrosis even in patients with cirrhosis (Dienstag, Schiff et al. 1999; Kweon, Goodman et al. 2001; Hadziyannis, Tassopoulos et al. 2003; Malekzadeh, Mohamadnejad et al. 2004). Nucleos(t)ide therapy is effective in decompensated chronic hepatitis B and as a consequence there has been a reduction in the number of liver transplants performed worldwide for hepatitis B related end-stage liver disease (Yao, Terrault et al. 2001; Fontana, Keeffe et al. 2002; Australia & New Zealand Liver Transplant Registry 2007; Kim, Benson et al. 2007). It has been estimated that the liver failure-related morbidity and mortality may be reduced by 80% in chronic hepatitis B by successful oral antiviral therapy (Toy, Veldhuijzen et al. 2009).

A major shortcoming of nucleos(t)ide analogue therapy is the frequent requirement for long-term therapy. Although for HBeAg positive patients HBeAg seroconversion represents a potential endpoint of treatment, this occurs only ~20% of patients after one year (Dienstag 2009). Furthermore, HBeAg negative patients predictably relapse even if HBV viral load has been suppressed to undetectable levels for more than a year (Marcellin, Chang et al. 2003; Hadziyannis, Tassopoulos et al. 2005; Hadziyannis, Tassopoulos et al. 2006). Therefore, once nucleos(t)ide therapy is instituted, most HBeAg positive and all HBeAg negative patients will require long-term therapy.
In the context of chronic therapy, the development of nucleos(t)ide drug resistance has become the major challenge confronting physicians managing patients with chronic hepatitis B (Ghany and Doo 2009). Drug resistance usually leads to reactivation of liver injury, compromises the beneficial effects of therapy, and may be associated with hepatic flares or potentially life-threatening hepatic decompensation (Lok, Lai et al. 2003; Liaw, Sung et al. 2004). Furthermore, the development of nucleos(t)ide drug resistance limits future treatment options due to significant cross-resistance between available agents (Lok, Zoulim et al. 2007). This problem is compounded when resistance develops to multiple agents and these patients are amongst the most difficult to treat. This thesis contains the results of a study into the use of tenofovir disoproxil fumarate, a newer anti-HBV nucleotide analogue, in patients with established multi-drug resistance.

A number of specific definitions are used to describe the emergence of drug resistance (Lok, Zoulim et al. 2007; Zoulim and Locarnini 2009). Primary treatment failure (or non-response) is characterized by an inability of nucleos(t)ide analogue to reduce serum HBV DNA by $>1 \log_{10} \text{IU/mL}$ with 12 weeks of treatment (or alternatively by an inability to achieve a $>2 \log_{10} \text{IU/mL}$ reduction with 24 weeks of treatment). Secondary treatment failure is often termed virological breakthrough and is defined as an increase in viral load $> 1 \log_{10}$ above nadir (after an initial virological response) despite compliance with ongoing therapy. Biochemical breakthrough is defined as a loss of biochemical response and is usually preceded by virological breakthrough. Genotypic resistance is defined as the detection of viral genetic sequence mutations known to be associated with decreased in vitro anti-viral activity.

The efficacy, safety and antiviral resistance profile of the currently available anti-HBV nucleos(t)ides is discussed below. The clinical endpoints achieved with these drugs are summarised in Table 2, Table 3, and Figure 4.

1.6.3.1 Lamivudine

Lamivudine was the first oral anti-HBV agent to be approved, however it has largely been replaced by newer drugs which possess better resistance profiles.
<table>
<thead>
<tr>
<th>Endpoint (at 1 year)</th>
<th>Lamivudine</th>
<th>Adefovir</th>
<th>Telbivudine</th>
<th>Entecavir</th>
<th>Tenofovir</th>
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<td>3.5</td>
<td>6.4</td>
<td>6.9</td>
<td>6.2</td>
</tr>
<tr>
<td>Virological Response (undetectable by PCR)</td>
<td>44%</td>
<td>21%</td>
<td>60%</td>
<td>67%</td>
<td>76%</td>
</tr>
<tr>
<td>Biochemical Response (ALT normalization)</td>
<td>66%</td>
<td>48%</td>
<td>77%</td>
<td>68%</td>
<td>68%</td>
</tr>
<tr>
<td>Histological Response (↓ activity, ⇔ fibrosis)</td>
<td>59%</td>
<td>53%</td>
<td>65%</td>
<td>72%</td>
<td>74%</td>
</tr>
<tr>
<td>Serological Response (HBeAg seroconversion)</td>
<td>22%</td>
<td>12%</td>
<td>23%</td>
<td>21%</td>
<td>21%</td>
</tr>
<tr>
<td>HBsAg loss at 1 year</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>1.7%</td>
<td>3%</td>
</tr>
</tbody>
</table>

**Table 2: Properties of oral anti-HBV agents in HBeAg positive patients.**

See text for references.

Lamivudine is nucleoside analogue of cytidine (specifically the (-) enantiomer of 2',3'-dideoxy 3'-thiacytidine). It is phosphorylated to lamivudine tri-phosphate which competes with cytidine to interrupt DNA reverse transcription and DNA replication (by obligate chain termination), and hence inhibit viral replication (Severini, Liu et al. 1995).

Lamivudine is effective in reducing HBV viral loads in HBeAg positive and HBeAg negative patients, including those who are previous interferon non-responders or those with advanced liver disease including decompensated cirrhosis. Lamivudine is administered orally, usually 100mg once daily in adults, though
may be dose adjusted in the setting of renal impairment or for children, and is very well tolerated (Lok and McMahon 2007).

In HBeAg positive patients with elevated ALT levels, 12 months of lamivudine therapy leads to HBeAg seroconversion in 17% of patients vs. 6% of controls (Dienstag, Schiff et al. 1999). This is inferior to the results achieved with pegylated interferon for the same duration of therapy. Extending lamivudine therapy for >4 years increases the rate of HBeAg sero-conversion to ~50% but at the cost of the emergence of drug resistance in up to 65% of patients (Lok, Lai et al. 2003; Chang, Lai et al. 2004). HBeAg seroconversion induced by lamivudine therapy persists after cessation of lamivudine in 50-80% of patients, although this appears to be dependant on the duration of consolidation lamivudine therapy, hepatitis genotype and patient age (Chien, Yeh et al. 2003; Ryu, Chung et al.

<table>
<thead>
<tr>
<th>Endpoint (at 1 year)</th>
<th>Lamivudine</th>
<th>Adefovir</th>
<th>Telbivudine</th>
<th>Entecavir</th>
<th>Tenofovir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fall in HBV DNA Log_{10} copies/mL</td>
<td>4.5</td>
<td>3.9</td>
<td>5.2</td>
<td>5.0</td>
<td>6.2</td>
</tr>
<tr>
<td>Virological Response (undetectable by PCR)</td>
<td>72%</td>
<td>63%</td>
<td>88%</td>
<td>90%</td>
<td>93%</td>
</tr>
<tr>
<td>Biochemical Response (ALT normalization)</td>
<td>71%</td>
<td>77%</td>
<td>77%</td>
<td>78%</td>
<td>76%</td>
</tr>
<tr>
<td>Histological Response (↓ activity, ↔ fibrosis)</td>
<td>61%</td>
<td>69%</td>
<td>65%</td>
<td>70%</td>
<td>72%</td>
</tr>
<tr>
<td>HBsAg loss at 1 year</td>
<td>&lt;1%</td>
<td>0%</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>0%</td>
</tr>
</tbody>
</table>

**Table 3: Properties of oral anti-HBV agents in HBeAg negative patients.**

See text for references.
In HBeAg positive patients with normal ALT levels, rates of HBeAg seroconversion are poor, being only 10% at 1 year (Perrillo, Lai et al. 2002).

**Figure 4: Resistance to oral anti-HBV agents with successive years of therapy**

See text for references.
In HBeAg negative patients, 12 months of lamivudine suppresses HBV replication to undetectable levels (by PCR based assays) in 60-70% of patients (Chan, Leung et al. 2000). Unfortunately this suppression is not durable and usually lost following cessation of treatment. In one study, 85% of patients who had achieved an undetectable viral load during treatment developed recurrence of viremia at 12 months post-treatment (Santantonio, Mazzola et al. 2000).

Lamivudine is safe and confers a significant survival benefit in patients with end stage liver disease, including those with decompensated cirrhosis awaiting liver transplant. One study described 75% transplant-free survival at 18 months for lamivudine treated decompensated cirrhotics compared to 0% in untreated historical controls (Yao, Terrault et al. 2001). The improvement in liver function may takes >6 months to become apparent (Villeneuve, Condrey et al. 2000). A prospective randomised controlled study of 651 Asian patients with advanced fibrosis or cirrhosis was terminated prematurely when an interim analysis showed significantly less progression to decompensated disease or HCC in the lamivudine treated group (Liaw, Sung et al. 2004).

By far the major disadvantage of lamivudine therapy is the development of resistance with long-term therapy. Genotypic resistance is most commonly conferred by substitution of methionine with valine or isoleucine in the tyrosine-methionine-aspartate-aspartate (or ‘YMDD’) motif of the HBV polymerase. This occurs at amino acid position 204 and is thus labelled rtM204V/I (Lok, Zoulim et al. 2007). In vitro studies show rtM204I/V virus to be >100-fold less susceptible to the effects of lamivudine than wild-type virus (Chin, Shaw et al. 2001). In comparison to wild-type virus, rtM204I/V- mutated virus is replication defective, however replication fitness is restored by further compensatory mutations particularly rtL180M either alone or in combination with rtV173L and rtL80I (Ono-Nita, Kato et al. 1999; Ono, Kato et al. 2001). The lamivudine resistance mutations rtM204I/V show cross-resistance to the other L-nucleoside agents which share a similar molecular structure, including telbuvudine (rtM204I only), emtricitabine, and clevudine (see below) (Ghany and Doo 2009). Less frequently,
primary resistance to lamivudine may be conferred by rtA181T/V mutation which also confers resistance to adefovir dipivoxil (Yeh, Chien et al. 2000; Yatsuji, Noguchi et al. 2006).

The rate of lamivudine resistance with long-term lamivudine therapy is >65% with 5 years of continuous therapy and this high rate of resistance has led to it being supplanted by other, newer, oral anti-HBV agents with better resistance profiles (Lok, Lai et al. 2003; Chang, Lai et al. 2004). Risk factors for lamivudine resistance include duration of treatment, viral load at the commencement of therapy, and persistence of significant viral replication whilst on therapy (Yuen, Sablon et al. 2001; Lok, Lai et al. 2003).

Lamivudine resistance is associated with inferior clinical outcomes. For example, in the Liaw study of patients with advanced HBV-related liver disease, patients receiving lamivudine who developed resistance (49% of 436 patients over a median of 32.4 months) were less protected than those who did not. In this study, 7% of patients with lamivudine resistance reached the combined endpoint of hepatic decompensation, hepatocellular cancer or death from liver disease compared to <1% of patients without lamivudine resistance (Liaw, Sung et al. 2004).

Effective long-term lamivudine therapy (complete response without breakthrough) has been shown to prevent progression of fibrosis and leads to regression of fibrosis and even cirrhosis in some patients (Dienstag, Goldin et al. 2003). Survival is also improved. Five-year disease-specific transplant-free survival is greater with lamivudine than no treatment, and major event-free survival (combined endpoint of death, decompensation, HCC or liver transplant) is improved in patients with a persisting lamivudine-induced virological response (Papatheodoridis, Dimou et al. 2005).

1.6.3.2 Adefovir dipivoxil

Adefovir dipivoxil was the second oral anti-HBV agent to achieve widespread clinical use. This drug is a diester prodrug of adefovir, which is phosphorylated to form adefovir diphosphate, an acyclic nucleotide analogue of adenosine
monophosphate (Marcellin, Chang et al. 2003). Adefovir diphosphate interferes with both the reverse transcriptase and polymerase activity of the HBV polymerase/RT, causing obligate DNA chain termination and thereby inhibition of viral replication. Adefovir dipivoxil in a dose of 10mg daily is safe with prolonged usage. Renal dysfunction is the major significant side-effect and this has been observed in 3% of patients after 5 years of therapy (Hadziyannis, Tassopoulos et al. 2006). The dose is adjusted (dose interval extended) in the setting of renal impairment.

In 515 HBeAg positive patients, 12 months of treatment with adefovir dipivoxil 10mg daily produced a 53% histological response, 48% biochemical response, 21% virological response (suppression of DNA to below 400 copies/mL using a PCR based assay), and 12% HBeAg seroconversion rate compared with 25%, 16%, 0% and 6% respectively in those on placebo (Marcellin, Chang et al. 2003). The median change in viral load over the courses of this study was -3.52 log_{10} copies/mL (-0.55 log_{10} copies/mL with placebo). No adefovir resistance mutations were noted.

In the corresponding study of 185 HBeAg negative patients, 12 months of adefovir was associated with a 64% histological response, 51% virological response (again using a PCR based assay with a threshold of 400 copies/mL,) and 72% biochemical response compared with 33%, 0% and 29% respectively in those on placebo (Hadziyannis, Tassopoulos et al. 2003). The median change in viral load over the course of this study in adefovir treated patients was -3.91 log_{10} copies/mL (as opposed to -1.35 log_{10} copies/mL with placebo). Again no genotypic resistance was identified with 12 months of therapy. As with lamivudine, in HBeAg negative patients cessation of adefovir therapy is usually associated with relapse of viraemia. Only 8% of patients switched after 48 weeks of adefovir to placebo had sustained suppression of viral replication <1000 copies/mL with 48 further weeks of follow-up (Hadziyannis, Tassopoulos et al. 2005).

In general adefovir dipivoxil is considered a less potent drug and inferior to lamivudine in achieving short-term biochemical and virological responses. The
median fall in viral load with 12 months of adefovir in HBeAg positive patients is $-3.5 \log_{10}$ copies/mL as opposed to $-5.5 \log_{10}$ copies/mL with lamivudine (Dienstag 2008). Furthermore, approximately 25% of patients have a suboptimal response to adefovir (a fall in viral load of $<2 \log_{10}$ copies/mL) (Fung, Chae et al. 2006). The major advantage of adefovir therapy over lamivudine therapy is its improved resistance profile. However, its lack of potency has seen it superseded by other agents as first line therapy and its major use has been in lamivudine resistance HBV.

Genotypic resistance develops less frequently (or later) with long-term adefovir therapy than with lamivudine. In a long-term follow-up study of 70 HBeAg negative patients treated with adefovir, genotypic resistance did not occur within 1 year of treatment and at 2, 3, 4 and 5 years resistance was observed in 3%, 11%, 18% and 29% of patients, respectively (Hadziyannis, Tassopoulos et al. 2006). This compares to a 70% rate of resistance observed with 4 years of lamivudine therapy (Lai, Dienstag et al. 2003). As a result of this improved resistance profile, although less potent than lamivudine, adefovir better maintains antiviral efficacy with prolonged therapy. In the Lai study, the number of patients with undetectable HBV DNA (<1000 copies/mL) peaked at 2 years (80%) and fell only slowly with further follow-up to 77%, 73% and 67% at 3, 4 and 5 years respectively (Lai, Dienstag et al. 2003).

The two major primary adefovir resistance mutations include an asparagine to threonine substitution at amino acid position 236 (rtN236T) and an alanine to valine or threonine substitution at amino acid position 181 (rtA181T/V) (Angus, Vaughan et al. 2003; Lacombe, Ollivet et al. 2006). Genotypic resistance to adefovir only confers a modest decrease in sensitivity in vitro (<10-fold change in EC$_{50}$) however (possibly due to limited antiviral potency) viral rebound and clinical deterioration has been observed in patients with these mutations (Fung, Andreone et al. 2005; Lok, Zoulim et al. 2007). The rtA181T mutation has been recognised as a potential mode of cross-resistance between adefovir and lamivudine. In addition, it has been recently observed that, due to overlapping HBV reading frames, the rtA181T mutation can encode a premature stop codon in
the surface protein at amino acid position 172 (denoted sW172) which may lead to a negative effect on HBV particle secretion even in the presence of multiple other viral quasi-species (Warner and Locarnini 2008).

Despite the recent recognition of a shared resistance pathway (rtA181T/V) and therefore the potential for cross-resistance, adefovir is usually effective in the setting of established lamivudine resistance. In one study, 59 patients with genotypic lamivudine-resistant HBV, ALT greater than twice the upper limit of normal and viral load >6 log_{10} copies/mL despite ongoing lamivudine were randomized to switch to adefovir, add adefovir to lamivudine or to continue lamivudine alone (Peters, Hann Hw et al. 2004). Median change from baseline viral load was -3.59 and -4.04 log_{10} copies/mL in the adefovir and adefovir/lamivudine groups respectively, but unchanged in the lamivudine monotherapy group. Approximately 50% of the patients randomized to receive adefovir (with or without lamivudine) had a biochemical response compared to 5% in the group randomized to lamivudine alone.

Adefovir is also effective in patients with advanced liver disease including liver transplant candidates with lamivudine resistant HBV. In an open-label compassionate use study of adefovir in 324 waitlisted or post-liver transplant patients with lamivudine resistant chronic hepatitis B, adefovir was shown to be safe and effective (Schiff, Lai et al. 2003). Viral load dropped by 4.1 log_{10} copies/mL when it was used pre-transplant and by 4.3 log_{10} copies/mL when used to treat post-transplant HBV recurrence. 81% of pre-transplant patients and 34% of post-transplant patients treated for 48 continuous weeks achieved a viral load of <400 copies/mL. Child-Pugh-Turcotte score improved in >90% of both cohorts and an increase in creatinine >0.5g/dL above baseline was observed in only 2% of pre-transplant and 6% of post-transplant patients, respectively.

Risk factors for the development of adefovir resistance include suboptimal viral suppression and sequential switch from lamivudine monotherapy to adefovir monotherapy rather than to lamivudine/adefovir combination therapy (Fung, Chae et al. 2006; Hadziyannis, Tassopoulos et al. 2006; Lee, Suh et al. 2006). The
role of combination lamivudine and adefovir therapy is discussed in more detail in Chapter 1.6.4.

1.6.3.3 Entecavir

Entecavir is an orally administered anti-HBV agent which is phosphorylated intracellularly into an analogue of deoxyguanidine triphosphate (dGTP). The drug blocks hepatitis B replication at three points, specifically reverse transcription of negative sense DNA, synthesis of the positive sense DNA strand, and base priming of the HBV polymerase (Lok and McMahon 2007). Entecavir is well tolerated, with adverse event rates similar to those of lamivudine observed in two phase III licensing trials (Chang, Gish et al. 2006; Lai, Shouval et al. 2006). In pre-clinical rodent studies, entecavir in dosage 30- to 40-fold greater than that used in humans was associated with lung adenomas, brain gliomas and hepatocellular cancers however this has not been found in higher species including rabbits and dogs (Dienstag 2008). Entecavir is administered in a dosage of 0.5mg daily (1.0 mg daily to lamivudine-resistance patients) and dosage is adjusted in the setting of renal dysfunction.

In 715 HBeAg positive patients, 48 weeks of entecavir 0.5mg daily resulted in a significantly better biochemical (68% vs. 60%), virological (67% vs. 36%, using PCR assay) and histological (72% vs. 62%) responses than with lamivudine 100mg daily (Chang, Gish et al. 2006). HBeAg seroconversion was similar in both groups (21% vs. 18%). The median fall in HBV DNA with entecavir was -6.9 log_{10} copies/mL vs. -5.4 log_{10} copies/mL with lamivudine. Patients who achieved both an HBeAg seroconversion and a virological response at 48 weeks had therapy ceased, and 77% of entecavir-treated patients and 72% of lamivudine-treated patients maintained this HBeAg seroconversion after a further 24 weeks of off-treatment follow-up (Gish, Lok et al. 2007). Further treatment to 96 weeks in patients who had not achieved an HBeAg seroconversion but had achieved some measure of virological response resulted in more pronounced superiority of entecavir over lamivudine: a cumulative 74% vs. 37% virological response by PCR assay and 79% vs. 68% biochemical response. Again the proportion of patients achieving HBeAg seroconversion was similar, 11% with entecavir and 12% with
lamivudine. Importantly there was no entecavir resistance observed during 96 weeks of therapy.

In the corresponding trial in HBeAg negative patients, 48 weeks of entecavir was again shown to be superior to lamivudine in achieving biochemical (78% vs. 71%), virological (90% vs. 72%) and histological (70% vs. 61%) responses (Lai, Shouval et al. 2006). Median fall in HBV DNA with entecavir was -5.0 log_{10} copies/mL vs. -4.5 log_{10} copies/mL with lamivudine.

In addition to being more potent than lamivudine, entecavir also has a much more favourable resistance profile with long-term therapy. After five years of entecavir, genotypic resistance is observed in 1.2% of previously nucleos(t)ide naive patients (Tenney, Rose et al. 2009). More than one distinct mutations is required for the development of genotypic entecavir resistance: rtM204V + rtL180M are required in addition to one or more of rtI69T, rtM250I/V, rtS202G/C/I or rtT184S/A/I/LG/C/M (Hoofnagle, Doo et al. 2007). The requirement for multiple mutations has been termed a 'high genetic barrier to resistance' and is thought to explain the low rates of genotypic entecavir resistance with chronic therapy.

It can be seen that entecavir resistance is heavily dependant on the lamivudine resistance mutation rtM204V with the compensatory mutation rtL180M. In isolation, rtI69T, rtS202G/C/I and rtT184S/A/I/LG/C/M have little effect on entecavir EC_{50}, whereas rtM250I/V has only a modest effect (a nine-fold reduction in sensitivity) (Lok, Zoulim et al. 2007). It is therefore not surprising that the durability of response to entecavir is severely compromised by pre-existing lamivudine resistance. With each successive year of entecavir therapy in patients with lamivudine resistant HBV the rate of entecavir resistance increases progressively to reach 51% at five years (6%, 15%, 36% and 47% at 1, 2, 3, and four years of therapy, respectively) (Tenney, Rose et al. 2009).

Although prior lamivudine resistance predisposes towards entecavir resistance, entecavir suppresses lamivudine resistant virus. In a study of 284 patients with lamivudine resistance randomized to receive 0.1, 0.5 or 1.0 mg of entecavir or 100 mg lamivudine, entecavir 1.0 mg was associated with a significantly better
virological response than 0.5 mg with 24 weeks of therapy (Chang, Gish et al. 2005). As a consequence 1.0 mg daily has been adopted as the standard entecavir dose for treatment of lamivudine resistant HBV. However, the risk of the subsequent development of entecavir resistance makes entecavir monotherapy a less than ideal treatment for lamivudine resistant HBV.

Entecavir is also effective against adeovir-resistant virus in vitro and has also been reported effective clinically although there are no large studies (Villeneuve, Durantel et al. 2003; Fung, Chae et al. 2006).

Entecavir is only partial inhibitor of HIV replication (and is not licensed for this purpose) and furthermore the development of a lamivudine-resistant HIV variant has been observed during entecavir therapy in the setting of HIV/HBV co-infection (McMahon, Jilek et al. 2007).

1.6.3.4 Tenofovir disoproxil fumarate

Tenofovir disoproxil fumarate is a prodrug that is cleaved by diester hydrolysis to form tenofovir which is then phosphorylated to form tenofovir diphosphate (Ghany and Doo 2009). Tenofovir is an acyclic nucleotide analogue of adenosine monophosphate, very similar to adeovir from which it differs by a single methyl group. In Australia tenofovir has been used since 2001 for the management of HIV, however it was only approved for use against hepatitis B in November 2008 and was listed for subsidy under the PBS for this indication from December 2009. Although structurally very similar and equipotent to adeovir in vitro, tenofovir is significantly less nephrotoxic than adeovir and therefore can be administered in much higher dosage (300 mg vs. 10 mg daily) (Lok and McMahon 2007). In the setting of renal dysfunction tenofovir dosage is reduced or the dose interval extended.

The phase III licensing trial of tenofovir involved 375 HBeAg positive patients and 266 HBeAg negative patients randomized in a 2:1 ratio to 48 weeks of therapy with tenofovir or adeovir (Marcellin, Heathcote et al. 2008). In HBeAg positive patients, tenofovir was superior in achieving virological (76% vs. 13%) and biochemical (68% vs. 54%) responses, but not histological response (74% vs.
68%, p=NS) or HBeAg seroconversion (21% vs. 18%, p=NS). In HBeAg positive patients, the median fall in HBV DNA was -6.18 log_{10} copies/mL with tenofovir compared to -3.93 log_{10} copies/mL with adefovir. In HBeAg negative patients tenofovir was again significantly better than adefovir at achieving a virological response (93% vs. 63%), however there was no significant difference in histological (72% vs 69%, p=NS) or biochemical response (76% vs. 77%, p=NS). In HBeAg negative patients, the median fall in viral load was -4.55 log_{10} copies/mL with tenofovir compared to -4.07 log_{10} copies/mL with adefovir. Importantly no resistance was observed with 48 weeks of therapy, and no patient had an increase in serum creatinine >0.5g/dL (44.2 μmol/L) above baseline.

Two-year efficacy data for both HBeAg positive and negative patients has been reported in abstract form (Heathcote, Gane et al. 2008; Marcellin, Buti et al. 2008). At the end of the initial 48-week study, all adefovir-treated patients were switched to tenofovir. In addition, patients with persistent HBV replication (load >400 copies/mL) at week 72 had emtricitabine added to ongoing tenofovir. In HBeAg positive patients treated with tenofovir for the full 96 weeks a virological response was noted in 89% (77% by intention-to-treat analysis), cumulative HBeAg seroconversion in 27%, and HBsAg loss occurred in 6% (some of whom seroconverted to HBsAb). In HBeAg negative patients treated with tenofovir for the full 96 weeks, HBV DNA fell to <400 copies/mL in 90% of patients (98% by intention-to-treat analysis) however there was no HBsAg loss. In both HBeAg positive and negative patients switched from adefovir to tenofovir at 48 weeks, tenofovir showed significant additional antiviral effect. For example, all 35 HBeAg negative patients initially randomized to adefovir who had a viral load >400 copies/mL at 48 weeks achieved an undetectable viral load of <400 copies/mL with a further 48 weeks of therapy with tenofovir.

Two- and three-year resistance surveillance data has also been published for tenofovir in abstract form (Snow-Lampart, Chappell et al. 2008; Snow-Lampart, Chappell et al. 2009). No genotypic resistance to tenofovir has been identified thus far in naïve patients. Tenofovir genotypic resistance via the rTA194T mutation has been described in two HIV-HBV co-infected patients and confirmed
in vitro (Sheldon, Camino et al. 2005). This mutation may decrease the replication fitness of hepatitis B however it has been suggested that this is restored in the presence of basic core-promoter or precore mutation as found in HBeAg negative chronic hepatitis B (Amini-Bavil-Olyaei, Herbers et al. 2009).

Although chemically similar to adefovir, tenofovir shows modest cross-resistance (4.5-fold increase in EC50) to the primary adefovir resistance mutation rtN236T in vitro and even less cross-resistance (1- to 3-fold change in EC50) to rtA181T/V (Locarnini, Shaw et al. 2004; Brunelle, Jacquard et al. 2005; Lok, Zoulim et al. 2007). There is limited clinical experience with the use of tenofovir in adefovir resistant HBV. An initial retrospective study of 20 patients with established lamivudine and adefovir resistance was encouraging. In this study, 19 of 20 patients achieved an HBV DNA of <400 copies/mL after a median of 3.5 months of tenofovir therapy (one patient had persistent HBV DNA >400 copies/mL despite 15 months of tenofovir) (van Bommel, Zollner et al. 2006). Subsequent retrospective reports from the same group have suggested that tenofovir remains effective for patients with a sub-optimal response to adefovir in general, its efficacy may be reduced in the presence of genotypic adefovir resistance (van Bömmel, Trojan et al. 2007; van Bömmel, Feucht et al. 2008). This has been supported by another retrospective report of 13 patients (Tan, Degertekin et al. 2008).

Tenofovir disoproxil fumarate therefore shares similar potency to entecavir as well as a similar resistance profile with equivalent length of therapy. Tenofovir has no cross-resistance with the L-nucleosides and the drug remains active against lamivudine-resistant hepatitis B (van Bommel, Zollner et al. 2006). Tenofovir has also been reported to be effective in the setting of entecavir resistance (Leemans, Niesters et al. 2008). In contrast to adefovir, primary non-response is not observed with tenofovir therapy (Dienstag 2009). The drug is well tolerated and safe with long-term use (though renal function must be monitored and there have been isolated cases of Fanconi syndrome) (Verhelst, Monge et al. 2002; Lok and McMahon 2007). As a consequence of all these properties, tenofovir promises to be an important therapeutic option in the
management of chronic hepatitis B not only in treatment naïve patients but also those with pre-existing resistance to lamivudine or entecavir as well as patients who have not responded optimally to adefovir therapy.

In Chapter two of this thesis I describe a prospective study of the use of tenofovir disoproxil fumarate in 60 patients all with prior failure of lamivudine, a sub-optimal response to adefovir despite >24 weeks of therapy and a high rate of genotypic resistance. At the time of writing, this is the largest prospective study of the use of tenofovir in this difficult to treat population of patients.

### 1.6.3.5 Telbivudine

Telbivudine has been licensed for the treatment of hepatitis B however in Australia it is not subsidised under the Pharmaceutical Benefits Scheme (PBS) at the time of writing and therefore has not had widespread use in this country. Telbivudine is an L-enantiomer of thymidine which is believed to cause chain termination and is a potent inhibitor of HBV replication *in vitro* (Dienstag 2009). Telbivudine is as well tolerated as lamivudine and is administered orally in a dose of 600 mg daily (Lai, Leung et al. 2005). Telbivudine is unique among anti-HBV nucleos(t)ides in that it does not select for HIV resistance.

The phase III licensing trial of telbivudine, which involved 1370 patients of whom 921 were HBeAg positive and 446 HBeAg negative, compared outcomes with telbivudine vs. lamivudine (Lai, Gane et al. 2007). In HBeAg positive patients, 48 weeks of telbivudine was significantly superior to lamivudine in achieving virological (60 vs. 40%) and histological (65% vs. 56%) but not biochemical (77% vs. 75%) or serological responses (23% vs. 22%). The median fall in HBV DNA with telbivudine in HBeAg positive patients was \(-6.4 \log_{10} \text{copies/mL}\) compared with \(-5.5 \log_{10} \text{copies/mL}\) with lamivudine. Extension of treatment to 96 weeks demonstrated a small but significantly better rate of ALT normalization with telbivudine (67% vs. 61%) but rates of HBeAg seroconversion were similar with the two drugs (34% vs. 29%, \(p>0.05\)) (Liaw, Gane et al. 2009). In HBeAg negative patients, telbivudine was superior in achieving virological response (88% vs. 71% at 1 year, 79% vs. 53% at 2 years) but at 48 weeks rates of ALT normalization and
histological responses were similar. At 96 weeks there was a slightly better biochemical response with telbivudine but again histological responses were similar. The median fall in HBV DNA in HBeAg negative patients with telbivudine was $-5.2 \log_{10}$ copies/mL compared to $-4.4 \log_{10}$ copies/mL with lamivudine.

Although modestly more potent than lamivudine, the major limitation of telbivudine therapy is a relatively high rate of primary resistance and cross-resistance with lamivudine. After 1 and 2 years of telbivudine therapy resistance occurs in 4.4% and 21.6% of HBeAg positive patients and 2.7% and 8.6% of HBeAg negative patients (Lai, Gane et al. 2007). Telbivudine resistance occurs by the primary lamivudine resistance mutation rtM204I (rtM204V has not been observed in the context of telbivudine therapy). Although the rate of development of telbivudine resistance is approximately two thirds of that observed with lamivudine, the availability of agents with better resistance profiles has discouraged the widespread use of this drug.

1.6.3.6 Other Agents

Emtricitabine is phosphorylated intra-cellularly to emtricitabine triphosphate which is an L-nucleoside analogue of cytosine. It is approved for use in HIV therapy but not yet for hepatitis B. Emtricitabine seems to have similar antiviral properties and resistance profile to lamivudine (Lim, Ng et al. 2006).

Clevudine is another L-nucleoside analogue (of pyrimidine) which is effective in lowering hepatitis B viral loads both in vitro and in animals of hepatitis B infection. In animal (woodchuck) models, clevudine had the property of durable HBV suppression after cessation of therapy that was associated with a reduction in the covalently closed circular DNA (cccDNA) (Summers and Mason 2004).

Clevudine is well tolerated however it has not been shown to be superior to currently approved anti-HBV agents (Yoo, Kim et al. 2007; Yoo, Kim et al. 2007). Furthermore eradication of cccDNA has not been observed in clinical studies (Dienstag 2009). Resistance to clevudine via rtM204I mutation has been observed within 1 year of treatment (Ko, Kwon et al. 2009). Clevudine therefore shares cross-resistance with all other L-nucleosides (lamivudine, telbivudine and
emtricitabine). Clevudine therapy has recently been associated with the development of myopathy in a proportion of patients and this has led to a halt in current trials with this drug (Kim, Oh et al. 2009).

### 1.6.4 Combination therapy

There are several arguments for the use of combination therapy in the treatment of chronic hepatitis B. Basic principles of infectious disease therapy in chronic infections encourage the simultaneous use of multiple agents, preferably with a different mechanism of action, to maximise anti-microbial effect and minimise the development of resistance. There are multiple examples of combination therapy resulting in better clinical outcomes. Combination highly active anti-retroviral therapy (HAART) has revolutionized the management of HIV infection as it is far more effective and more durable than monotherapy. Standard treatment of chronic hepatitis C infection entails combination pegylated interferon and ribavirin. Examples of combination therapy in non-viral diseases include Mycobacterium tuberculosis and Helicobacter pylori where three or four agents are typically used.

Despite these convincing arguments, evidence for benefit of combination treatment in hepatitis B has been very slow to emerge. Trials of pegylated or standard interferon in combination with lamivudine consistently showed better on-treatment viral suppression with combined therapy however sustained off-treatment responses were no better with combination therapy than with interferon alone (Marcellin, Lau et al. 2004; Janssen, van Zonneveld et al. 2005; Lau, Piratvisuth et al. 2005). There have been no large trials published of newer nucleos(t)ides in combination with interferon. There is one randomised prospective trial of combination lamivudine and adefovir dipivoxil versus lamivudine alone in nucleos(t)ide naïve patients (Sung, Lai et al. 2008). This trial involved 115 HBeAg positive patients treated for two years. The fall in median viral load at 1 year was similar in both groups, however at two years the fall in HBV DNA with combination therapy was superior to that with lamivudine alone (-5.22 vs. -3.14 log_{10} copies/mL). The reason for this change is that 15% of patients in the combination therapy group had developed genotypic lamivudine resistance
(rtM204I/V) at 2 years compared to 43% of patients in the lamivudine monotherapy group. This higher rate of genotypic lamivudine resistance was associated with a higher rate of virological breakthrough. Only one patient in the combination group developed genotypic adefovir resistance (via rtN236T mutation). Another small study has found similar results comparing combination adefovir plus emtricitabine versus adefovir alone for two years (Hui, Zhang et al. 2008). Combination therapy was associated with better virological response (-5.30 vs. -3.98 log_{10} copies/mL), and less genotypic emtricitabine resistance (in fact; none) than would have been expected on the basis of emtricitabine monotherapy trials in which 13% of patients developed emtricitabine resistance after only one year of therapy (Lim, Ng et al. 2006). Another study showed no benefit of combination lamivudine and telbivudine over telbivudine alone (Lai, Leung et al. 2005).

There is more data to support the benefit of combination therapy in the setting of established lamivudine resistance. Specifically, addition of adefovir to ongoing lamivudine is clearly superior to switching from lamivudine monotherapy to adefovir monotherapy in the prevention of future adefovir resistance. This was first suggested in a study of 43 patients who had adefovir therapy either added to lamivudine, switched to from lamivudine, or de novo (Fung, Chae et al. 2006). Six of 43 patients became adefovir resistant, none of whom had been treated with ongoing combination adefovir and lamivudine (p=0.01). Subsequently a prospective study found no adefovir resistance in 28 patients with add-on therapy for a median of 40 months in comparison to 3 of 14 patients who developed resistance to adefovir after sequential monotherapy (Rapti, Dimou et al. 2007). In another prospective study of 145 patients in whom adefovir was added at the onset of lamivudine resistance the cumulative rate of genotypic adefovir resistance with combination therapy was 4% at 4 years (Lampertico, Vigano et al. 2007). Combination adefovir and lamivudine was also found to be effective in a retrospective Australian study of 161 patients with lamivudine resistant HBV in which no cases of adefovir resistance occurred with ongoing combination therapy (van der Poorten, Prakoso et al. 2007). Furthermore, in patients who had a finite duration of cross-over combination therapy prior to
adefovir monotherapy, later adefovir resistance was more likely if cross-over combination treatment had been less than three months duration. Finally, follow-up of 467 pre- and post-liver transplant patients who received adefovir subsequent to lamivudine failure (98% with YMDD motif mutation) found only 4 cases of adefovir resistance, none of which occurred in the setting of ongoing combination treatment (Snow, Thibault et al. 2005).

Unfortunately, as a consequence of the chronology of drug availability, antiviral characteristics and previous prescribing patterns (dictated by either best clinical evidence at the time and in Australia the conditions for subsidy under the PBS) there are a large cohort of patients with previous failure of lamivudine and inadequate response to adefovir. In this setting both entecavir and combination lamivudine/adefovir are less than ideal therapeutic options due to a high likelihood of resistance. In Chapter two of this thesis I investigate the use of tenofovir in this difficult to treat population of patients.
1.7 Post-transplant hepatitis B prophylaxis

The early experience of liver transplantation for hepatitis B virus related disease was that the majority of patients developed recurrent infection in the early post-transplant period. This resulted in rapidly progressive liver injury, early graft loss, and markedly reduced survival (Davies, Portmann et al. 1991; Todo, Demetris et al. 1991; O’Grady, Smith et al. 1992). These poor outcomes lead a number of groups to question the role of transplantation in HBsAg positive patients, especially those with active viral replication who are at highest risk of disease recurrence. However, over the last two decades, the development of highly effective prophylaxis has almost eliminated the problem of HBV recurrence. As a consequence, long-term post-transplant survival in HBsAg positive patients now equals that of most other patient groups.

The most widely used prophylaxis has been long-term therapy with combination IV hepatitis B immune globulin (HBIG) and lamivudine. However this treatment is very expensive and its administration is inconvenient for patients. This has lead to the development of a range of alternate strategies for the use of HBIG aimed at reducing costs and improving tolerability.

1.7.1 HBIG monotherapy

Hepatitis B immune globulin was the first agent to show efficacy in preventing HBV recurrence. Limited duration (<12 months) HBIG therapy (10,000 IU IV at liver transplant followed by 10,000 IU IV daily for 8 days post-transplant and then at variable intervals, to maintain trough HBsAb (or anti-HBs) titres >100 IU/L) delayed, but did not prevent, graft re-infection (Muller, Gubernatis et al. 1991). The 1993 European multi-centre retrospective study demonstrated that if treatment was continued indefinitely, intravenous HBIG reduced the actuarial risk of recurrence from 74% to 36% at three years and, as a consequence, significantly improved survival (Samuel, Muller et al. 1993). Importantly, this
treatment was relatively ineffective in patients with HBV cirrhosis and detectable HBV DNA in serum (by hybridization assay) with more than 90% developing recurrent infection compared to 29% of those who were HBV DNA negative (Samuel, Bismuth et al. 1991). It is important to note, however, that the HBV DNA assays used in these early studies had a cut-off for detection of approximately $10^5$ copies/mL and thus many of the HBV DNA negative patients would have detectable HBV DNA by modern quantitative assays. Recurrence rates were also much lower in the presence of HDV (17%), and in those with fulminant hepatitis B (no recurrence); both conditions in which HBV DNA levels are usually low or undetectable at the time of transplant (Samuel, Muller et al. 1993). Thus, the efficacy of this therapy appeared to be highly dependent on the pre-transplant viral load.

In the original European studies, variable dose intervals were used with the aim of maintaining trough HBsAb concentrations >100 IU/L. The putative explanation for the relative ineffectiveness of this therapy in HBV DNA positive recipients was that there is increased clearance of HBsAb from serum in patients with high circulating viral loads, and this resulted in a failure to maintain protective HBsAb titres (McGory, Ishitani et al. 1996; Sawyer, McGory et al. 1998). This problem was partially overcome by using higher doses of HBig. The administration of fixed doses of 10,000 IU of HBig per month (which commonly achieves trough HBsAb levels of >500IU/L) or variable HBig dosing adjusted to keep HBsAb >500IU/L for the first six months post-transplant, reduced recurrence rates in HBV DNA positive patients to 20-30% (McGory, Ishitani et al. 1996; Terrault, Zhou et al. 1996; Sawyer, McGory et al. 1998). However, the use of such high doses of HBig (up to 200,000 IU in the first year) adds greatly to costs.

1.7.2 Lamivudine monotherapy

The early results using lamivudine as monotherapy to prevent recurrence were promising, with a re-infection rate of just 10% at 12 months post-transplant reported in the initial study (Grellier, Mutimer et al. 1996). However, 50% had developed recurrence by three years (Mutimer, Pillay et al. 1999). Similarly, Perillo et al reported 41% recurrence at three years in patients who received
lamivudine alone for post-transplant hepatitis B prophylaxis (Perrillo, Wright et al. 2001). These results were not surprising given that in non-immunosuppressed patients, resistance to lamivudine emerges at a rate of 15-20% per year as a result of selection of lamivudine resistant mutations in the YMDD motif of the HBV polymerase (Lai, Dienstag et al. 2003). As with HBIG, the risk of recurrence in patients receiving lamivudine monotherapy is most closely related to HBV load prior to its initiation: at three years post-transplant, patients with detectable HBV DNA by hybridization assay (equating to an HBV DNA of approximately $10^{5-6}$ copies/mL) at the initiation of lamivudine pre-transplant, had recurrence rates of 60% compared with 0% in patients who were HBV DNA negative (Mutimer, Pillay et al. 1999; Perrillo, Wright et al. 2001). In most studies, HBV DNA positive patients have commenced lamivudine treatment at the time of listing in order to reduce the level of viral replication at transplantation. Importantly, this can lead to improvements in liver function that in some patients may be sufficient to allow removal from the waiting list (Villeneuve, Condrey et al. 2000; Yao, Terrault et al. 2001). However, prolonged lamivudine therapy exposes patients to the risk of developing lamivudine resistance pre-transplant (Perrillo, Wright et al. 2001).

1.7.3 High dose IV HBIG and lamivudine

HBIG and lamivudine have very different mechanisms of action. It was therefore likely that in combination these two agents would have a number of independent effects which increased antiviral activity and reduced the risk of recurrence (Markowitz, Martin et al. 1998). Indeed, the lowest reported rates of post transplant hepatitis B recurrence (0 -10%) have been achieved with combined lamivudine and HBIG (see Table 4) (Markowitz, Martin et al. 1998; Han, Ofman et al. 2000; Marzano, Salizzoni et al. 2001; Rosenau, Bahr et al. 2001; Steinmuller, Seehofer et al. 2002). As a result, for the last decade, combination therapy with conventional high dose IV HBIG and lamivudine (or other nucleosid(t)e) has been the most widely used standard of care for preventing post-transplant HBV recurrence.
## Table 4: Post-transplant HBV recurrence using high dose HBIG/LAM

<table>
<thead>
<tr>
<th>HBIG Protocol (reference)</th>
<th>Patients (n)</th>
<th>DNA + pre-NA</th>
<th>Follow-up</th>
<th>Recurrent HBV</th>
</tr>
</thead>
<tbody>
<tr>
<td>80-100,000 IU in 1st month, then 10,000 IU/month. Markowitz et al. 1998.</td>
<td>14</td>
<td>36%</td>
<td>7%</td>
<td>12.7 months</td>
</tr>
<tr>
<td>80,000 IU 1st month, then 10,000 IU/month. Han et al. 2000.</td>
<td>59</td>
<td>34%</td>
<td>27%</td>
<td>15 months</td>
</tr>
<tr>
<td>46,500 IU in 1st month, then 5,000 IU/month. Marzano et al. 2001.</td>
<td>26</td>
<td>100%</td>
<td>27%</td>
<td>30 months</td>
</tr>
<tr>
<td>40,000 IU in 1st week, then aim HBsAb &gt;500 IU/L 7 days, then aim HBsAb &gt;100 IU/L.</td>
<td>21</td>
<td>52%</td>
<td>24%</td>
<td>21 months</td>
</tr>
<tr>
<td>Rosenau et al. 2001.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10,000 IU/day until HBsAb &gt;1,000 IU/L, then aim HBsAb &gt;100 IU/L. Rosenau et al. 2001.</td>
<td>19</td>
<td>100%</td>
<td>47%</td>
<td>NA</td>
</tr>
<tr>
<td>80,000 IU in 1st month, then 2,000 IU aim HBsAb &gt;100 IU/L. Seehof er et al. 2001.</td>
<td>17</td>
<td>100%</td>
<td>29%</td>
<td>25 months</td>
</tr>
<tr>
<td>10,000 IU daily until HBsAg cleared, then aim HBsAb &gt;100 IU/L. Steinmuller et al. 2002.</td>
<td>51</td>
<td>100%</td>
<td>NA</td>
<td>35 months</td>
</tr>
</tbody>
</table>

LT: liver transplant; HBIG: hepatitis B immune globulin; LAM-R: lamivudine resistance; NA: not assessable. HBsAb: Hepatitis B surface antibody. HBsAg: Hepatitis B surface antigen.
In most centres lamivudine is begun pre-transplant with the aim of reducing viral load in the peri-transplant period. IV HBIG is given at a dose of 10,000 IU daily for the first post-operative week and subsequently either at a fixed dose of 10,000 IU/month or variable dosing to maintain trough HBsAb titres >100 IU/L (Markowitz, Martin et al. 1998; Han, Ofman et al. 2000; Marzano, Salizzoni et al. 2001; Rosenau, Bahr et al. 2001; Roche, Feray et al. 2003). Some centres have targeted HBsAb titres of >500 IU/L for 3-6 months post-transplant in HBV DNA positive patients. In contrast to the experience with HBIG or lamivudine monotherapy, all of these various combinations are highly effective, regardless of the peri-transplant viral load (Han, Ofman et al. 2000; Rosenau, Tillmann et al. 2001).

However, there are a number of problems with this treatment. Patients receiving prolonged lamivudine therapy whilst on the liver transplantation waiting list may develop resistance and this can precipitate worsening liver failure. Post-transplant HBV recurrence rates are also increased in patients who have lamivudine resistant HBV at the time of transplant (see table 1) (Rosenau, Bahr et al. 2001; Rosenau, Tillmann et al. 2001; Seehofer, Rayes et al. 2001). Furthermore, combination HBIG/lamivudine regimes using fixed IV HBIG dosing are very expensive with an estimated cost of >$100,000 in the first year post-transplant and >$50,000 yearly thereafter (Dan, Wai et al. 2006).

1.7.4 Alternative combination IV HBIG/lamivudine regimes

The expense and inconvenience of long-term IV HBIG/lamivudine therapy has lead to the development of a number of strategies for reducing IV HBIG use. Several groups have attempted to individualise HBIG doses by allowing for inter-patient variability in HBIG elimination kinetics. Di Paolo et al estimated a >50% saving in cost using ‘on demand’ administration of 2000 IU (aiming for a trough HBsAb of >100 IU/L) in comparison to regular administration of 5,000 IU/month (Di Paolo, Tisone et al. 2004). In another study, 200 IU/Kg HBIG was administered daily for one week post-transplant. Until 6 months post-transplant, 2000IU HBIG
was given when the HBsAb titre was <100 IU/L, but thereafter doses were only given when the HBsAb was <10IU/L. There was no recurrence at median 30 months (range 7-73), despite a mean HBIG use of only 464 IU/month in years subsequent to the first post-transplant year (Takaki, Yagi et al. 2007). This suggests that in the presence of lamivudine it may not be necessary to achieve HBsAb levels of >100IU to minimise recurrence.

1.7.4.1 Low dose IM HBIG and lamivudine

A number of studies have shown that the use of IV HBIG is not necessary to maintain effective HBsAb titres and that in equivalent doses, IM HBIG has similar kinetics and produces roughly equivalent trough concentrations to IV dosing but at less cost (Hooman, Rifai et al. 2008). The largest reported experience with the use of IM HBIG prophylaxis comes from investigators in Australia and New Zealand, where IV HBIG preparations are not available. In 2000, it was reported that IM HBIG in combination with lamivudine prevented recurrence in 31 of 32 HBsAg positive liver transplant recipients with a median follow-up of 18.4 months post-transplant (Angus, McCaughan et al. 2000). All HBV DNA positive patients commenced lamivudine at transplant listing and no patient developed lamivudine resistance. These promising results were notable given that the amount of HBIG used (IM 800 IU/day for seven days and then IM 400-800 IU/month thereafter) was <10% of that used in standard high-dose IV HBIG protocols: approximately 15,000 IU in the first year vs. 150-200,000 IU. Trough HBsAb levels observed during follow-up with this regimen ranged from 27-181 IU/L. Importantly, prior to the initiation of lamivudine pre-transplant, 50% of the patients in this study were HBV DNA positive by hybridization assay and therefore at high risk of recurrence.

Long-term results using this protocol have recently been published. In 147 patients HBsAg positive patients transplanted over eight years, the actuarial rate of HBV recurrence was 4% at five years (Gane, Angus et al. 2007). Prior to commencement of lamivudine pre-transplant, 31% were HBeAg positive and 85% had detectable serum HBV DNA (PCR assay, LLOD 300 copies/mL) with a median viral load of $6 \log_{10}$ copies/mL. Over the same period, eight patients with
lamivudine resistance were transplanted using combination low dose IM HBIG, lamivudine and adefovir and none developed recurrence. On multivariate analysis the only factor associated with HBV recurrence–free survival was HBV viral load prior to lamivudine (p=0.004).

Similar results using IM HBIG and lamivudine have been reported by other groups, however, most have used larger doses or have targeted specific HBsAb levels (see Table 5). Karademir et al administered IM 4000 IU HBIG at transplant, IM 2000 IU HBIG daily until an HBsAb of 200 IU/L was reached, then IM 1200-2000 IU when HBsAb levels fell below 100 IU/L (Karademir, Astarcioglu et al. 2006). Although the mean total HBIG dose using this protocol was 34,000 IU in the first year post-transplant (compared to ~15,000 IU in the Australasian protocol), in subsequent years this decreased to ~5000 IU (compared to 9,600 IU in the Australasian protocol). At a median 16 months post-transplant, 2 of 35 patients developed recurrence, however, both had lamivudine resistance pre-transplant and were transplanted prior to the availability of adefovir or other antiviral agents effective against lamivudine-resistant HBV. IM HBIG has also been used as long-term maintenance therapy following initial therapy with high doses of IV HBIG for the first week post-transplant (IV 10,000 IU HBIG daily for 1 week) (Ferretti, Merli et al. 2004). In this study, doses of 1200 IU IM HBIG were given in an attempt to maintain HBsAb >100 IU/L. Over a mean of 20 months (range 7.8-43) post-transplant, only one of 28 patients developed recurrence and this was attributed to non-compliance. The mean HBsAb achieved was 62.3 IU/L. This protocol reduced the estimated cost of HBIG after the first post transplant years by 90% compared to conventional fixed high dose therapy.

A recent large retrospective Chinese study reported a higher rate of recurrence with combined low dose IM HBIG lamivudine prophylaxis than in previous studies (Zheng, Chen et al. 2006). 16 of 114 patients (14%) in this study developed recurrence at a mean of 15.8 months (range 8-36) post-transplant. The likely explanation is that only 15 of 114 patients commenced lamivudine prior to their transplant, despite approximately one third being high-risk patients with pre-transplant HBV DNA levels greater than 10⁵ copies/mL. Not surprisingly, most
of the recurrence occurred in these patients. These findings suggest that in patients with high levels of HBV replication, the efficacy of low dose HBIG therapy may be lessened if the viral load is not reduced with pre-transplant antiviral therapy.

<table>
<thead>
<tr>
<th>HBIG Protocol (reference)</th>
<th>Patients (n)</th>
<th>DNA+ pre-NA</th>
<th>Follow-up</th>
<th>Recurrent HBV</th>
</tr>
</thead>
<tbody>
<tr>
<td>IM 800 IU at LT and daily for 1 week, then IM 800 IU monthly Angus et al. 2000.</td>
<td>32</td>
<td>97%</td>
<td>NA</td>
<td>18.4 months</td>
</tr>
<tr>
<td>IV 80,000 IU in 1st week, then IM 1200 IU aim HBsAb &gt;100 IU/L Ferretti et al. 2004.</td>
<td>23</td>
<td>48%</td>
<td>13%</td>
<td>20 months</td>
</tr>
<tr>
<td>IM 4000 IU at LT, IM 2000 IU/day until HBsAb &gt;200 IU/L, then aim HBsAb &gt;100 IU/L Karademir et al. 2006.</td>
<td>35</td>
<td>51%</td>
<td>14%</td>
<td>16 months</td>
</tr>
<tr>
<td>IM 2,000 IU at LT, then IM 800 IU daily for 6 days, weekly for 3 weeks, then monthly Zheng et al. 2006.</td>
<td>114</td>
<td>NA (99 LAM post-LT only)</td>
<td>31%</td>
<td>15.8 months</td>
</tr>
<tr>
<td>IM 800 IU at LT and daily for 6 days, then IM 800 IU monthly Gane et al. 2007.</td>
<td>147</td>
<td>85%</td>
<td>&lt;50%</td>
<td>61 months</td>
</tr>
</tbody>
</table>

**Table 5: Post-transplant HBV recurrence using low dose HBIG/LAM**

LT: liver transplant; HBIG: hepatitis B immune globulin; LAM-R: lamivudine resistance; NA: not assessable. HBsAb: Hepatitis B surface antibody.
The excellent results achieved with very low doses of HBig/lamivudine therapy challenge conventional thinking about the mechanism of action of HBig and the doses and target serum levels of HBsAb required to optimise protection against recurrence. The therapeutic premise behind the original use of high doses of IV HBig monotherapy was that it prevented graft infection by clearance of circulating virions by antigen-antibody binding, that entry into allograft hepatocytes via a putative HBsAg receptor blockade was blocked, or that virion secretion was inhibited by HBig (Schilling, Ijaz et al. 2003). However, Roche et al demonstrated that nearly half of all patients treated with high dose IV HBig who were HBsAg negative at ten years post-transplant had detectable HBV DNA in serum, liver or peripheral blood mononuclear cells (Roche, Feray et al. 2003). Similarly, HBV DNA can be detected in more than 80% of allograft livers in patients who have remained serum HBsAg and HBVDNA negative under combination high dose IV HBig and lamivudine prophylaxis (Hussain, Soldevila-Pico et al. 2007).

Thus, neither high dose IV HBig nor combination HBig/lamivudine prophylaxis prevent graft infection. This explains the need for prophylaxis to be maintained life-long in most patients. However, the experience with very low dose IM HBig therapy suggests that in the presence of lamivudine or other powerful inhibitors of HBV replication, relatively low levels of HBsAb are required to prevent the emergence of resistant mutants and progression from low-level infection to overt disease recurrence (Terrault, Zhou et al. 1996).

1.7.4.2 HBig cessation

Several authors have investigated whether it is feasible to stop HBig following an initial successful period of prophylaxis with combination HBig/lamivudine. In the largest prospective study, 29 patients who were HBV DNA negative at liver transplant received HBig/lamivudine for one month post-transplant and were then randomized to continue combination prophylaxis or change to lamivudine monotherapy (Buti, Mas et al. 2007). The early results were promising, with no recurrence recorded in either group at 18 months. However, with longer follow-up, 15-20% of patients switched to lamivudine monotherapy have viral
breakthrough due to lamivudine resistance (Buti, Mas et al. 2003; Buti, Mas et al.
2005). The previous experience with lamivudine monotherapy prophylaxis
suggests that the risk of resistance following cessation of HBIG is likely to be
considerably lower in patients with low HBV DNA levels (<10⁵ copies/mL) prior
to commencing lamivudine therapy (Mutimer, Pillay et al. 1999; Perrillo, Wright
et al. 2001).

Other authors have used active post-transplant HBV vaccination with the aim of
achieving a durable endogenous HBsAb response thus eliminating the need for
continued HBIG. Two small studies reported that between 60% and 80% of
patients achieved an HBsAb titre of >10 IU/L following cessation of HBIG and
active immunization with 1-3 courses of triple vaccination with recombinant IM
HBV vaccine (Sanchez-Fueyo, Rimola et al. 2000; Albeniz Arbizu, Barcena
Marugan et al. 2003). However, other studies using a very similar vaccination
protocol have failed to replicate these findings (Angelico, Di Paolo et al. 2002; Lo,
Liu et al. 2005).

The most successful trial of post-transplant HBV vaccination achieved HBsAb
titres of >500 IU/mL in 80% of patients (Bienzle, Gunther et al. 2003). Potential
reasons for these very impressive results may be that HBIG was continued during
the course of vaccination (possible leading to greater immune stimulation), and
that two vaccine adjuvants were used to potentiate vaccine immunogenicity.
However, other studies using other adjuvants (Rosenau, Hooman et al. 2004) and
concomitant HBIG administration (Rosenau, Hooman et al. 2007), have failed to
replicate these results and this approach, although attractive, remains
experimental.

Another option to which may allow for the cessation of HBIG is replacing it with
another nucleos(t)ide analogue. The aim of this strategy is to eliminate both the
cost and inconvenience of HBIG whilst avoiding an unacceptable risk of HBV
recurrence. Chapter three of this describes a randomized prospective study of the
substitution of adefovir/lamivudine in place of low-dose IM HBIG/lamivudine in
patients at least 12 months post-liver transplant without evidence of recurrence.
1.7.5 Other nucleos(t)ide analogues and HBIG

The development of a range of new oral inhibitors of HBV replication has greatly expanded the therapeutic options available for both the prevention and treatment of post-transplant HBV recurrence. These drugs have also altered the natural history of HBV disease. Indeed, many studies have shown that successful antiviral therapy can halt the progression of HBV disease, leading to clinical stabilization and even reversal of liver failure (see section 1.6.3). As a result, the number of transplants being performed for HBV related liver failure is falling and HBsAg positive patients are now more likely to be listed for transplant due to the development of hepatocellular cancer.

The high rate of resistance with prolonged lamivudine therapy makes it a far from ideal drug for use in patients awaiting liver transplantation, as failure to maintain suppression of HBV replication may precipitate liver decompensation and increases the risk of HBV recurrence post-transplant. This has become of greater concern as waiting times for liver transplant have increased.

New drugs with lower rates of resistance have largely replaced lamivudine as first line therapy for chronic HBV, and these drugs clearly should also supplant lamivudine in the liver transplant setting. The newer drugs with which there has been greatest clinical experience in the treatment of chronic hepatitis B are adefovir (see section 1.6.3.2) and entecavir (see section 1.6.3.3). Entecavir is the more potent of the two, and long-term studies have shown that resistance to this drug occurs in just 1.2 % of patients after five years of continuous therapy (Tenney, Rose et al. 2009). Adefovir resistance rates are significantly higher and several studies have reported primary non-response rates to the standard 10mg dose of adefovir of 25% or more (Fung, Chae et al. 2006). Thus monotherapy with entecavir appears to be a more attractive candidate than adefovir for use in the transplant setting.

As primary non-response and combination resistance appear to be extremely uncommon when adefovir added to lamivudine, this is has been the treatment of choice in patients who present for transplant with established lamivudine
resistance (Lampertico, Marzano et al. 2006). In patients with lamivudine resistance entecavir is a less than ideal choice, as the presence of lamivudine resistance mutations predispose to subsequent entecavir resistance (Tenney, Rose et al. 2009). In contrast, a recent study suggested adefovir is very effective in preventing HBV recurrence post transplant in patients with lamivudine resistance (with or without the use of HBIG) (Schiff, Lai et al. 2007). Unfortunately, data were not available from this study to determine how many patients received lamivudine/adefovir or adefovir alone. Although only recently approved for use in HBV, tenofovir also has considerable potential for use in HBV prophylaxis is tenofovir (see 1.6.3.4). Tenofovir is also highly effective against lamivudine resistant virus (van Bommel, Zollner et al. 2006).

Thus entecavir, adefovir/lamivudine and tenofovir are all candidates to replace lamivudine for the treatment of HBsAg positive patients awaiting liver transplant and for use in combination with HBIG post-liver transplant. All have potent antiviral activity in treatment naïve patients and their use should lead to further improvements in post-liver transplant outcomes in HBsAg positive patients. In patients with pre-existing lamivudine resistance, adefovir/lamivudine (or adefovir/entecavir) or tenofovir/lamivudine therapy are logical treatment options. Of course individualised combinations will be required to provide optimal protection in patients with more complex patterns of drug resistance. These new drugs have largely replaced lamivudine in the treatment of chronic hepatitis B, however, their safety and efficacy in the transplant setting is yet to be determined.

It is a tantalising prospect that potent oral antiviral therapy, either alone or in combination, will provide a sufficient barrier to the emergence of resistant mutants to allow for the elimination of HBIG prophylaxis altogether. Chapter four of this thesis describes a prospective study of combination adefovir and lamivudine initiated at waitlisting for HBsAg positive liver transplantation candidates. This regime was continued pre- and post-transplant without prolonged (> one week) HBIG therapy. This is the first prospective study of combination lamivudine and adefovir used in this manner.
Chapter 2. Tenofovir rescue therapy

following failure of both lamivudine

and adefovir in chronic hepatitis B.
2.1 Introduction

The development of safe, effective, oral anti-viral agents has revolutionised the management of chronic hepatitis B. Although suppression of viral replication with these drugs has been shown to alter the natural history of the disease and diminish the incidence of complications, long-term efficacy is compromised by the emergence of drug resistance (Dienstag, Schiff et al. 1999; Kweon, Goodman et al. 2001; Malekzadeh, Mohamadnejad et al. 2004). This is a major problem with lamivudine, with up to 70% of patients developing resistance after 4 years of treatment (Chang, Lai et al. 2004).

Adefovir dipivoxil has been widely used in the treatment of patients with lamivudine-resistant HBV (Peters, Hann Hw et al. 2004). However, up to 25% of patients treated with adefovir for lamivudine-resistant HBV fail to achieve a satisfactory virological response and 30% of naïve patients develop adefovir resistance at 5 years (Hadziyannis, Tassopoulos et al. 2006). Until recently, the treatment options for patients who fail sequential lamivudine then adefovir monotherapy have been limited. One option is entecavir, a drug that has an excellent resistance profile in treatment naïve patients even with long-term therapy (Tenney, Rose et al. 2009). This profile is compromised in the presence of lamivudine resistance, and in this setting genotypic entecavir resistance is observed in >50% of patients at five years. Another option is tenofovir disoproxil fumarate (DF), an acyclic nucleotide analogue and structural congener of adefovir that has potent antiviral activity against wild-type HBV (Marcellin, Heathcote et al. 2008). In treatment naïve patients, tenofovir DF durably suppresses HBV and drug resistance has not been encountered with up to 3 years of continuous therapy (Snow-Lampart, Chappell et al. 2009). Despite its structural similarity to adefovir, tenofovir DF inhibits adefovir-resistant HBV and it is also highly effective against lamivudine-resistant virus, suggesting that this drug may be an effective treatment for patients who have previously failed treatment with
lamivudine and adefovir (van Bommel, Zollner et al. 2006; van Bommel, de Man et al. 2010).

This chapter describes a prospective trial of tenofovir rescue therapy in patients with lamivudine resistant HBV who failed to achieve an adequate response to rescue therapy with adefovir. The study included both patients who had received adefovir as monotherapy and those who had received combination therapy with lamivudine.
2.2 Methods

This was a prospective, open label, multi-centre trial that recruited patients from eight tertiary referral hospitals in Victoria and New South Wales, Australia from September 2006 to December 2007. The relevant institutional ethics review committees approved the trial at each site and all subjects gave written informed consent prior to screening for the study.

Adult patients were considered for the study if they satisfied all of the following criteria: chronic hepatitis B (defined as detectable hepatitis B surface antigen (HBsAg) for at least 6 months); previous failure of lamivudine therapy due to the emergence of drug resistance; current treatment with adefovir dipivoxil (with or without lamivudine) for at least 24 consecutive weeks leading up to baseline; and the presence of significant persistent or breakthrough viremia. The threshold used to define significant viremia was 5 \( \log_{10} \) copies/mL in hepatitis B e antigen (HBeAg) positive patients and 4 \( \log_{10} \) c/mL in those who were HBeAg negative, equivalent to \( \sim 4.24 \log_{10} \) IU/mL and \( \sim 3.24 \log_{10} \) IU/mL, respectively. Patients remained eligible if they were receiving lamivudine and adefovir concurrently at screening but only if this regime had been in place and unchanged for at least 24 weeks. In addition, patients were required to have a creatinine clearance of \( >50 \) mL/minute (estimated by Cockcroft–Gault calculation), hemoglobin \( >8 \) g/dL, neutrophil count \( >1000/\text{mm}^3 \), and ALT not elevated to more than ten times the upper limit of normal.

Patients were excluded if they had previously used tenofovir DF; had recently (<6 months) used any other agent with anti-HBV activity other than adefovir or lamivudine; had coexisting hepatitis C, hepatitis D, or human immunodeficiency virus infection; had evidence of hepatocellular carcinoma; had decompensated liver disease or significant renal, cardiovascular, respiratory or neurologic co-morbidity; or were pregnant, breastfeeding or unwilling to use adequate contraception during the study. Patients potentially satisfying the inclusion and
exclusion criteria were offered inclusion in the study and then screened. If they continued to satisfy the inclusion criteria, patients began study medication at their baseline visit scheduled within 30 days of screening.

At the baseline visit, patients commenced treatment with 300 mg tenofovir daily (Gilead Sciences, East Melbourne, Victoria, Australia). The last dose of adefovir was taken the day prior to baseline. After baseline, tenofovir DF dosage was adjusted according to estimated creatinine clearance per the manufacturer’s guidelines. Those taking lamivudine and adefovir prior to baseline continued on lamivudine treatment in addition to tenofovir DF throughout the study. The remainder (i.e., those on adefovir monotherapy) received tenofovir DF monotherapy with the option of commencing lamivudine add-on therapy if they had a suboptimal response as defined by a viral load > 351 IU/mL (or 2.55 log10 IU/mL, the lower limit of detection of the screening VERSANT HBV DNA 3.0 bDNA assay (Siemens Healthcare Diagnostics, Tarrytown, NJ) at or beyond 24 weeks.

Patients were reviewed (physician assessment, physical examination, urine pregnancy test for females of childbearing potential, study medication accounting) at 4, 8 and 12 weeks and then at 12 weekly intervals until the conclusion of the study at 144 weeks. At each study visit, blood was taken for routine hematology and biochemistry, HBsAg and hepatitis B surface antibody (anti-HBs), HBeAg and anti-HBe and measurement of HBV DNA (including storage of serum at -70° C). In addition, baseline serum was tested for hepatitis B virus genotype and resistance sequence analysis was performed on the HBV polymerase gene. Resistance sequencing was also performed every 48 weeks during the study (in patients with sufficient viral load) or upon virological breakthrough (confirmed >1 log10 IU/mL increase in viral load from nadir).

The primary endpoint of the study was change in hepatitis B viral load expressed as both median fall in load from baseline and as a time weighted change from baseline (DAVG12). Secondary endpoints of the study included the proportion of patients achieving a viral load <15 IU/mL by the Abbott RealTime HBV assay (Abbott Molecular, Des Plaines, IL, USA), the incidence of virologic breakthrough or genotypic resistance, the incidence of HBeAg seroconversion, and safety,
including effects on renal function. This analysis of study outcomes was performed after 96 weeks of follow-up in all patients.

2.2.1 HBV assays

Viral markers (HBsAg, anti-HBs, HBeAg and anti-HBe) were measured by standard commercial immunoassays. Hepatitis B viral loads at screening, baseline and over the course the study were assessed by either the Siemens Healthcare Diagnostics VERSANT HBV DNA 3.0 bDNA assay or the Abbott RealTime HBV PCR assay according to the local reference laboratory. The lower limit of detection (LLOD) for these assays is quoted as 351 IU/mL and 15 IU/mL, respectively. Viral loads initially assayed by the bDNA assay with a result below the LLOD were repeated using the Abbott HBV DNA assay on stored serum. All results reported in copies/mL have been converted to IU/mL by the manufacturer’s recommended conversion factor.

HBV genotyping and sequencing of the polymerase/envelope region was performed in a single reference laboratory as follows. HBV DNA was extracted from 200 µl of serum using the QIAamp DNA MiniKit (QIAGEN, Melbourne, Australia) and eluted in a final volume 50 µl with the supplied elution buffer. For amplification of the HBV polymerase gene, a single primer set was used: sense primer 5’ CCT GCT GGT GGC TCC AGT TC (nt 1877-1996, numbering in accordance to HBV sequence described by Gan et al (Gan, Chu et al. 1987)) and antisense primer 5’ GCG TCA GCA AAC ACT TGG C (nt 2996-3014). Each reaction was carried out using 5 µl of the extracted DNA as template, 1.5 U of HotStar Taq polymerase (QIAGEN) 1 µmol/L of sense and antisense primers, 200 µmol/L each of deoxynucleoside triphosphates, 50 mmol/L KCl, 3.5mM MgCl, 10 mmol/L Tris-HCl (pH 8.3) and 0.01% gelatin. Amplification conditions were as follows: initial cycle of 94°C for 15 mins, 40 cycles of denaturation (94°C for 30 sec), annealing (57°C for 30 sec) and extension (72°C for 40 sec) followed by a final extension of 10 min. The PCR product was analysed by gel electrophoresis through 1.0% agarose and visualised by UV irradiation after staining with ethidium bromide. The amplified HBV polymerase/surface product was purified using an UltraClean PCR Clean-up Kit (MO BIO Laboratories, Carlsbad, CA) and directly sequenced
using Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) with the same primers as employed for the PCR. HBV consensus sequences were constructed using the DNA sequence analysis program Seqscape (Applied Biosystems). HBV genotype was determined using a web-based program, SeqHepB (Yuen, Ayres et al. 2007). Successful HBV genotyping and sequencing by this method requires a viral load of approximately $\log_{10} 2.55$ IU/mL (2000 copies/mL).

2.2.2 Statistical Methods

Continuous variables are expressed as median (range) unless specified otherwise. Categorical data are presented as number (percent). Time weighted average change in viral load (DAVGt) is calculated by dividing the area under the response-time curve from the first post-baseline measurement to the measurement at time $t$ by the time between those two measurements, and then subtracting the baseline viral load. $P$ values <0.05 were considered statistically significant, and the statistical test used is specified with the corresponding result.
2.3 Results

Between September 2006 and December 2007, 67 patients were screened for the study in 8 sites. Six patients were ineligible on screening (viral load below inclusion criteria) and one was lost to follow-up; 60 remaining patients were enrolled in the study and received tenofovir DF. The baseline (first day of study medication) characteristics of the study population are shown in Table 6.

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>HBeAg Status</th>
<th>Initial Study treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>positive</td>
<td>negative</td>
</tr>
<tr>
<td>Number of patients</td>
<td>60</td>
<td>40 (67%)</td>
<td>20 (33%)</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>48.5 (21-80)</td>
<td>44 (21-80)</td>
<td>52 (29-69)</td>
</tr>
<tr>
<td>Male gender (%)</td>
<td>46/60 (77%)</td>
<td>29/40 (73%)</td>
<td>17/20 (85%)</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>40/60 (66.7%)</td>
<td>30/40 (75%)</td>
<td>10/20 (50%)</td>
</tr>
<tr>
<td>European</td>
<td>9/60 (15%)</td>
<td>3/40 (7.5%)</td>
<td>6/20 (30%)</td>
</tr>
<tr>
<td>Other</td>
<td>11/60 (18.3%)</td>
<td>7/40 (17.5%)</td>
<td>4/20 (20%)</td>
</tr>
<tr>
<td>Median Baseline Viral Load log₁₀ IU/mL (range)</td>
<td>5.33 (2.81 - &gt;8)</td>
<td>5.43 (3.99 - &gt;8)</td>
<td>5.07 (3.12 - 7.8)</td>
</tr>
</tbody>
</table>

**Table 6: Baseline patient demographics and characteristics.**

TDF: tenofovir. LAM: lamivudine.
Forty-six (76.7%) patients were male and the median age was 48.5 years (range 21-80). Most (65%) of the patients were of Asian ethnicity, 15% were of European background and the remaining 20% were of Pacific Island, African, or Middle-Eastern background. The median viral load at baseline was $5.33 \log_{10}$ IU/mL (range 2.81 - 8.05 $\log_{10}$ IU/mL).

The majority of patients (66.7%) were HBeAg positive at baseline and 22 (36.7%) were receiving combination adefovir and lamivudine therapy immediately prior to baseline and therefore received combination tenofovir and lamivudine therapy from commencement of the study. When sub-grouped by HBeAg status or treatment with lamivudine at baseline, the sub-groups were well matched with the exception that there were fewer patients of European ethnicity in the HBeAg negative sub-group than in the HBeAg positive sub-group (3/40 versus 6/20, $p=0.048$ by Fisher’s exact test). Baseline viral load did not differ significantly between these sub-groups.

In accordance with the entry criteria, all patients had been exposed to lamivudine and adefovir. The median duration of drug exposure was 37.5 months for lamivudine (range 8.2-102.5) and 21.9 months (range 6-85.4) for adefovir. In addition, six patients had been previously treated with interferon (five with standard and one with pegylated interferon) for median 6.1 months (range 4-12), and three patients had received entecavir for median 9.1 months (range 8.1-30.4). No patient had received entecavir within 6 months of screening.

Genotyping and resistance sequencing was performed on baseline serum from 59 patients (one patient exited the study after 10 days (see below) and therefore stored baseline serum was not assayed). As expected with the preponderance of patients of Asian ethnicity, the most frequent genotype was C in 31 patients (52.5%), followed by D (25.4%), B (13.6%) and A (8.5%). The rtM204I/V mutation was detected in 20 patients (33.9%) at baseline. Sixteen of these 20 patients had compensatory rtL180M mutation and seven had a variety of other compensatory mutations including rtL80I, rtV173L, or rtT184S. The rt181T/V mutation was detected in 14 (23.7%) patients and seven (11.9%) patients had
rtN236T mutation (four harbouring both rtA181T/V and rtN236T). No other polymerase mutations were identified.

Median viral load at baseline did not differ significantly according to the presence of rt181T/V or rtN236T (either separately or in combination when compared to the remaining patients on study). The presence of the rtM204I/V mutation both alone and in combination with rtL180M was associated with a lower median baseline viral load compared to the remaining patients on study (median 4.97 versus 6.09 log_{10} IU/mL, p=0.011 and median 4.94 versus 6.07 log_{10} IU/mL, p=0.019; respectively, see Table 7). Median baseline viral load showed no association with HBV genotype.

2.3.1 Patient survival, withdrawal and follow-up.

All 60 patients enrolled in the study were alive after 96 weeks. One patient developed a rash within ten days of baseline and was withdrawn from the study and switched to alternative anti-HBV therapy. The following results do not include the withdrawn patient and minimum follow-up in the remaining patients is 96 weeks.

2.3.2 Effects of therapy on HBV DNA

The changes in viral load during the study both overall and for various subgroups is shown in Table 7 and illustrated in Figure 5, Figure 6 and Figure 7. The median change in viral load from baseline to week 12 was -2.86 log_{10} IU/mL. The median time weighted average change in viral load to week 12 (DAVG_{12}) was -2.20 log_{10} IU/mL. All patients had a primary response to therapy defined as a viral load reduction of >1 log_{10} IU/mL by 12 weeks (Zoulim and Locarnini 2009). With the exception of one subject, all had a >2 log_{10} IU/mL fall in viral load by 24 weeks of therapy (this was achieved at 36 weeks in the remaining patient who had received tenofovir and lamivudine from baseline). The median viral load continued to fall throughout the course of the study reaching -4.06 log_{10} at 96 weeks. To 96 weeks of therapy there were no significant differences in the median viral load change between HBeAg positive versus negative patients; patients receiving tenofovir versus those receiving tenofovir/lamivudine
combination; patients with HBV genotype A, B, C or D versus the remainder; or according to the presence or absence of baseline sequence mutations (specifically rtM204I, combination rtM204I & rtL180M, rtA181T/V, rtN236T, or combination rtN236T & rtA181T/V).

<table>
<thead>
<tr>
<th>Study treatment</th>
<th>Median Baseline VL</th>
<th>Median change in load from baseline to week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td><strong>All patients</strong></td>
<td></td>
<td>5.33</td>
</tr>
<tr>
<td><strong>Baseline HBeAg</strong></td>
<td>Positive n=39</td>
<td>5.43</td>
</tr>
<tr>
<td></td>
<td>Negative n=20</td>
<td>5.07</td>
</tr>
<tr>
<td><strong>TDF/LAM</strong> n=22</td>
<td>5.32</td>
<td>-1.87</td>
</tr>
<tr>
<td><strong>Baseline ADV-R</strong></td>
<td>rtA181T/V n=14</td>
<td>6.22</td>
</tr>
<tr>
<td></td>
<td>rtN236T n=7</td>
<td>6.07</td>
</tr>
<tr>
<td><strong>Baseline LAM-R</strong></td>
<td>rtM204I/V n=20</td>
<td>4.97</td>
</tr>
</tbody>
</table>

**Table 7: Median change in HBV Viral load over study.**

VL: viral load. DAVG_{12}: time weighted change in viral load from baseline to week 12. TDF: tenofovir disoproxil fumarate. LAM: lamivudine. LAM-R: genotypic LAM resistance. ADV-R: genotypic adefovir resistance. * These 16 patients are a subset of the 37 that began the study on TDF monotherapy, see text.
**Figure 5: Virological response: all patients**

Dotted graph denotes viral load median and 25th/75th percentiles. Shadowed area denotes proportion of patients achieving <15IU/mL.
Figure 6: Virological response: baseline adefovir resistance.

Individual patients graphed. Shadowed area denotes the proportion of patients that achieved <15 IU/mL in the relevant group. Top (Figure 6A): presence of rtA181T/V alone. Bottom (Figure 6B): presence of rtN236T alone or in combination with rtA181T/V. LLOD: lower limit of detection (15 IU/mL).
**Figure 7: Virological response by treatment.**

Dotted graph denotes viral load median and 25th/75th percentiles. Shadowed area denotes proportion of patients achieving <15 IU/mL. Top (Figure 7A): tenofovir from baseline. Middle (Figure 7B): tenofovir/lamivudine from baseline. Bottom (Figure 7C): Baseline tenofovir switched to tenofovir/lamivudine combination due to persistent viral replication at 24 weeks (a subset of 7A). LLOD: lower limit of detection (15 IU/mL).
Overall, 27/59 (45.8%) and 38/59 (64.4%) patients achieved an undetectable viral load <15 IU/mL with 48 and 96 weeks of therapy, respectively (Figure 5). As might be expected, persistent viral replication >15 IU/ml was associated with higher baseline viral load at all time points to week 96 (p<0.003 at weeks 12-48, p<0.017 at weeks 60-84, p=0.029 at week 96 by Fisher’s exact test, comparing patients with a baseline viral load either greater or less than or equal to the median baseline value). Persistent viral replication was associated with baseline HBeAg positive status up to 48 weeks (p=0.021 at 24 weeks, p=0.01 at 36 and 48 weeks) but not at later time points. There was no significant relationship between persistent viral replication >15 IU/mL and the presence or absence of substitutions conferring antiviral resistance at baseline (or combinations of substitutions as described above). The change in viral load over time for individual patients with either of the adefovir resistance mutations, rtA181T/V or rtN236T, is illustrated in Figure 6. The use of combination tenofovir and lamivudine therapy from baseline had no significant effect on persistence of viral replication >15 IU/mL (see Figure 7).

A suboptimal virological response, defined at commencement of the study as a persistent viral load >351 IU/mL at 24 weeks, occurred in 25 patients; nine of whom had received combination therapy from baseline. As per protocol, the remaining 16 patients had lamivudine added to tenofovir from 24 weeks. The change in median viral load for these patients (and the proportion who achieved a viral load <15 IU/mL) is illustrated in Figure 7C. There was no significant difference in median change in viral load from baseline through to 96 weeks in these 16 patients compared to those patients receiving combination therapy from baseline (p>0.25 at all time points by Mann-Whitney test). As expected, the requirement for lamivudine add-on therapy due to sub-optimal response after 24 weeks was associated with persistent viremia >15 IU/mL when compared to all patients receiving tenofovir/lamivudine from baseline (p<0.015 at 24-60 and at 96 weeks, p<0.05 at 72-84 weeks, see Figure 7A & Figure 7C). When these 16 patients with sub-optimal response to tenofovir monotherapy at 24 weeks were
compared to similar sub-optimal responders who had received combination therapy from baseline (nine patients) there was no difference in the proportion achieving an undetectable viral load with ongoing therapy beyond 24 weeks.

There were seven confirmed episodes of virological breakthrough observed in 5 patients during the study to 96 weeks, three of which (two in the same patient) are thought to have occurred in the context of poor compliance. Following five of these episodes of breakthrough the viral load declined below the previous nadir either spontaneously or with compliance to ongoing tenofovir. The other two episodes occurred at 84 weeks (near the end of the follow-up period). Viral load in both patients declined at 96 weeks however not beyond the previous nadir. In all episodes polymerase sequencing was performed and no unique polymerase resistance mutations were identified compared to baseline.

2.3.3 Sequence mutations on study

In four of 59 patients substitutions in the HBV polymerase emerged during the study. Three instances of this were observed at 48 weeks at which point viral load was >351 IU/mL. The substitution rtA181T was selected in one patient who had lamivudine added at 24 weeks, and rtN236T in another patient with pre-existing rtA181T substitution who had received tenofovir and lamivudine from baseline. Both patients achieved an undetectable viral load (<15 IU/mL) by week 96. In the third patient who had received additional lamivudine therapy from 24 weeks, both rtA181T and rtN236T substitutions emerged at 48 weeks and were still detected at 96 weeks with viral replication persistently >351 IU/mL. In the remaining patient rtA181T was detected at 96 weeks. This patient was treated with combination tenofovir and lamivudine from baseline and had never achieved a viral load <351 IU/mL. No other unique polymerase changes associated with nucleos(t)ide resistance were detected. (Warner and Locarnini 2008)

2.3.4 Biochemical and serological response

Median ALT fell during the course of the study from 49 IU/L at baseline (range 17-528 IU/L) to 35 IU/L (14-62 IU/L) at week 96. One HBeAg negative patient had an ALT greater than ten times the upper limit of normal at baseline (violating
study inclusion criteria) however ALT progressively fell in this patient during the study. Elevated ALT levels were present in 33/60 patients (55%) at baseline and this had normalised in 17/33 patients at 96 weeks (51.5% of those with initially elevated ALT).

Of the 39 HBeAg positive patients at baseline, two (5.1%) and four (12.8%) lost HBeAg at 48 and 96 weeks, respectively. None have seroconverted to anti-HBe positive. No patient has lost HBsAg or seroconverted to anti-HBs to 96 weeks.

2.3.5 Safety and side effects

Serum creatinine did not change significantly over the course of the study from a median of 80 μmol/L (range 48-116) at baseline to 83 μmol/L (range 49-122) at 48 weeks and 85 μmol/L (range 64-118) at 96 weeks. No patient had an increase in serum creatinine of >0.5 mg/dL (44.2 μmol/L). Tenofovir dosage interval was adjusted to alternate days in one patient between weeks 4 and 72 according to estimated creatinine clearance (latest follow-up in this patient 120 weeks). This patient had a baseline creatinine clearance of 51 mL/minute that was unchanged at latest follow-up (52 mL/minute).

Transient elevations in ALT to greater than two times the upper limit of normal were seen in 13/59 patients (22%) during the study however all such elevations subsequently resolved with one exception. One patient developed an elevated ALT to greater than ten times the upper limit of normal at 48 weeks in the context of using a herbal remedy. Viral load was undetectable (<15 IU/mL) at that time and a liver biopsy was performed showed histology consistent with a drug reaction. The herbal remedy was ceased, tenofovir therapy continued and ALT fell to ~2-3 times the upper limit of normal but has not normalised at 96 weeks.

Apart from the one patient who discontinued tenofovir due to rash after ten days, there were no other withdrawals from the study. There were no significant abnormalities in other laboratory parameters during the study and no serious adverse events considered related to study therapy.
2.4 Discussion

This study prospectively demonstrates that tenofovir DF produces significant suppression of HBV replication in patients with lamivudine resistance who have failed to respond adequately to adefovir. Despite a background of extensive prior treatment and the presence of clinical resistance to lamivudine and/or adefovir in a significant proportion of patients, none experienced a primary non-response to study drug and after 2 years of follow-up, none have developed persistent breakthrough or novel polymerase mutations. Importantly, viral load continued to fall throughout treatment in the majority of patients, with the median viral load reduction being -2.86, -3.75 and -4.03 log_{10} IU/mL at 12, 48 and 96 weeks of therapy. However, as might be expected, the virological response to tenofovir DF in this study appears to be inferior to that observed in treatment naïve patients. The key registration studies of tenofovir DF reported that 69% of 176 HBeAg positive and 91% of 250 HBeAg negative nucleos(t)ide naïve patients achieved a viral load of <169 copies/mL (approximately <30 IU/mL) after 48 weeks of tenofovir (Heathcote, Gane et al. 2008; Marcellin, Buti et al. 2008). For comparison in the current study 33.3% (13/39) of HBeAg positive and 70% (14/20) of HBeAg negative patients achieved a viral load <15 IU/mL (approximately <80 copies/mL) by 48 weeks (overall 27/59 or 45.8%).

In the current study, the effectiveness of tenofovir DF in achieving an undetectable viral load was also less than in previously published reports nucleos(t)ide experienced patients receiving tenofovir. A retrospective study of the response to tenofovir DF therapy in patients with viral breakthrough on lamivudine and persistent viral replication >10^4 copies/mL despite adefovir found 19/20 (95%) patients achieved an HBV load <400 copies/mL within a median of 3.5 months (van Bommel, Zollner et al. 2006). None of these patients had genotypic adefovir resistance. A subsequent larger retrospective report from the same group included 121 patients with persistent viral replication, of whom 105 had received lamivudine and 75 adefovir (van Bömmel, De Man et al. 2007). Twenty patients
were excluded due to poor compliance or genotypic adeovir resistance. Of the remaining 101 patients, 91% achieved a viral load <400 copies/mL after 48 week of therapy. In a separate report of 10 patients with genotypic adeovir resistance, only 2/10 (20%) achieved the same endpoint (van Bömmel, Trojan et al. 2007). In another small retrospective study, 11/13 (84.6%) patients (three with genotypic adeovir resistance) achieved a viral load <200 copies/mL although four had additional emtricitabine either from baseline or for initial suboptimal response (Tan, Degertekin et al. 2008). Finally, another study randomized 105 patients (of whom approximately 60% had been exposed to lamivudine) with sub-optimal response to no more than 96 weeks of adeovir to combination tenofovir DF/emtricitabine or tenofovir DF alone. Ten of these patients had baseline genotypic adeovir resistance (Berg, Moller et al. 2008; Berg, Moller et al. 2009). At 48 weeks, ~80% of patients in both treatment groups and 80% of patients with baseline genotypic adeovir resistance had achieved an HBV load <400 copies/mL. When the current study is analysed using an equivalent threshold of 400 copies/ml, 61% of patients achieved this endpoint by 48 weeks.

A possible explanation for the lower rate of virological response is an extensive prior exposure to lamivudine and adeovir and a high rate of genotypic resistance. The patients in the current study were heavily pre-treated, all having had prior lamivudine and adeovir for a median of 3 and 1.75 years, respectively. Furthermore, 17/59 (28.8%) patients had genotypic resistance to adeovir and 20 (33.9%) to lamivudine. This rate of baseline genotypic adeovir resistance is more than double that in previous reports of nucleos(t)ide experienced patients receiving tenofovir. Importantly the current study shows that despite this, with 96 weeks of follow-up there was ongoing suppression of viral replication in all patients and median viral load continued to fall in the group as a whole.

Both rtA181T and rtN236T confer reduced sensitivity to tenofovir in vitro (Qi, Xiong et al. 2007). It was previously observed in an interim analysis of this data that patients with baseline rtN236T mutation were more likely to have persistent viral replication quantifiable by the bDNA assay (LLOD of >351 IU/mL) at 48 weeks compared with those patients without rtN236T (p=0.042) (Patterson,
George et al. 2009). When a highly sensitive PCR-based assay was employed in the current study the proportion of patients infected with HBV with this substitution who achieved an HBV DNA <15 IU/mL was no longer significantly different to those without (p=0.43 at 48 weeks and 0.085 at 96 weeks). However, when the individual profiles were examined in patients with baseline rtN236T substitution, five of seven remained HBV DNA positive at 96 weeks and in three of these patients there was no appreciable fall in viral load beyond 36 weeks (Figure 7). Within this context, it is of considerable interest that adefovir resistant HBV variants were selected in 4 patients during the course of the study, and longer-term follow-up will be required to determine the clinical and virological sequelae of these changes (Warner and Locarnini 2008). A recent retrospective analysis of tenofovir therapy in patients with prior failure of adefovir and/or lamivudine also suggested a blunting of the response to tenofovir in the presence of adefovir resistance substitutions. In this study, patients with baseline genotypic adefovir resistance had a significantly lower rate of achieving a viral load <400 copies/mL when compared to the remainder of the cohort after a mean of 23 months treatment (52% versus 100%, respectively) (van Bommel, de Man et al. 2010). Although this study did not show such a difference it should be noted that the cut-off for detecting viral replication was significantly lower in the current study (15 IU/ml).

A very low proportion of patients were observed to achieve a serological endpoint. Only 10% of patients achieved HBeAg loss, none of whom seroconverted to anti-HBe positive (and there was no HBsAg loss or anti-HBs seroconversion). The likely explanation is that the patients in this study represented a poorly responding group who had previously failed to achieve serological endpoints with previous therapy.

Of note, the study design allowed for continuation of lamivudine from baseline in patients previously receiving adefovir/lamivudine, as well as for the addition of lamivudine after 24 weeks in patients with persistently significant viral replication on tenofovir DF alone. There was no difference over the study in the proportion of patients achieving an undetectable viral load or in the absolute fall in HBV DNA
between patients receiving tenofovir DF alone and those receiving combination lamivudine and tenofovir DF from baseline. Of the 37 patients initially receiving tenofovir monotherapy, 16 had persistent significant viral replication at 24 weeks and were switched to combination lamivudine and tenofovir. These 16 patients started with a higher viral load (median 6.91 vs. 5.32 log_{10} IU/mL) however the absolute fall in viral load in this group was similar to that observed in patients receiving combination therapy from baseline (see Table 7 and Figure 7). As expected, significantly more of these patients defined by sub-optimal response had persistent viral replication >15 IU/mL beyond 24 weeks. However, when compared to similarly defined sub-optimal responders receiving combination therapy from baseline, there was no difference in the proportion achieving an undetectable viral load (<15 IU/mL) between these two groups. Although the design of this study precludes conclusions being drawn regarding the optimum use of combination lamivudine and tenofovir there is certainly no clear evidence that additional lamivudine in this setting provides a significant supplemental antiviral effect. However one could argue from virological first principles that appropriate combination therapy should be employed where possible for the treatment of multidrug-resistant HBV in an attempt to reduce the risk of further drug resistance.

In conclusion, tenofovir DF appears safe and effective in patients with prior failure of lamivudine and suboptimal response to (or failure of) adefovir therapy. Despite the high prevalence of drug resistance mutations in the study population, no patient failed to respond to therapy or had persistent rebound/breakthrough of viral replication following the initiation of tenofovir DF therapy. Importantly, novel mutations conferring resistance to tenofovir were not observed. However, the efficacy of tenofovir in this heavily pre-treated population with a high rate of baseline genotypic adefovir resistance was inferior to that reported previously and further follow up is required to determine if this will be associated with subsequent virological breakthrough.
Chapter 3. A randomized study of adefovir dipivoxil in place of HBIG in combination with lamivudine as post-liver transplant hepatitis B prophylaxis.
3.1 Introduction

As discussed in Chapter 1.7, prior to the advent of effective post-transplant antiviral prophylaxis, liver transplantation for hepatitis B (HBV) related disease was usually followed by immediate HBV infection of the allograft (Davies, Portmann et al. 1991; Todo, Demetris et al. 1991; O’Grady, Smith et al. 1992). In the setting of post transplant immunosuppression, recurrent HBV infection was associated with severe liver injury, which often progressed rapidly to graft loss and death (Davies, Portmann et al. 1991; Harrison, Davies et al. 1993).

Over the past 15 years advances in antiviral prophylaxis have reduced the risk of HBV recurrence significantly. Long-term intravenous (IV) high dose hepatitis B immune globulin (HBIG) administration (Samuel, Muller et al. 1993; McGory, Ishitani et al. 1996) and lamivudine monotherapy (Grellier, Mutimer et al. 1996) both reduce recurrence rates from over 90% to less than 50% in patients who are HBV DNA positive prior to transplant. More recently, several studies have shown that these two approaches are synergistic and that combination lamivudine plus IV HBIG reduces recurrence rates to less than 5% at five years (Markowitz, Martin et al. 1998). Consequently, this combination is the accepted prophylaxis regimen at most transplant units. However, a major drawback of this therapy is that high dose intravenous HBIG is very expensive and the long-term IV administration of HBIG each month is inconvenient and unpleasant for patients.

Several strategies have been used to reduce the cost associated with long-term high dose IV HBIG administration. These include cessation of HBIG at some interval post transplant whilst continuing lamivudine (Dodson, de Vera et al. 2000; Naoumov, Lopes et al. 2001; Buti, Mas et al. 2007; Wong, Chu et al. 2007) vaccination of patients prior to cessation of HBIG (Sanchez-Fueyo, Rimola et al. 2000; Angelico, Di Paolo et al. 2002; Albeniz Arbizu, Barcena Marugan et al. 2003; Bienzle, Gunther et al. 2003; Lo, Liu et al. 2005; Rosenau, Hooman et al. 2007) and substitution of high dose IV HBIG preparations with low dose intramuscular (IM)
HBIG (McCaughan, Spencer et al. 1999; Angus, McCaughan et al. 2000; Gane, Angus et al. 2007).

Another possible approach is to replace HBIG with a direct antiviral drug such as adefovir dipivoxil since recent studies have demonstrated that the risk of virologic breakthrough during combination adefovir and lamivudine therapy is negligible (Lampertico, Vigano et al. 2007; van der Poorten, Prakoso et al. 2007). The combination of both drugs has been shown to be safe and effective in transplant recipients with lamivudine resistance (Perrillo, Hann et al. 2004; Schiff, Lai et al. 2007). This chapter describes a randomized study comparing the safety and efficacy of replacing HBIG with adefovir in combination with lamivudine versus continuing HBIG plus lamivudine in patients at least one year post-transplant for HBV related disease.
3.2 Methods

This was a randomized, open-label, multi-centre trial conducted at the Victorian (Austin Health, Melbourne), New South Wales (Royal Prince Alfred Hospital, Sydney), and New Zealand (Auckland City Hospital) liver transplant units between August 2004 and October 2006. The trial was approved by the appropriate institutional ethics review committees, and all subjects gave written informed consent prior to entry into the study.

All adult patients who were at least 12 months post-transplant for HBV-related disease and who were receiving protocol low dose IM HBIG and lamivudine prophylaxis without evidence of graft re-infection (defined below) were eligible. Patients were excluded if they had been transplanted for fulminant hepatic failure, had a serum creatinine of ≥180μmol/L, were HIV seropositive (or other significant co-morbidity), or were pregnant or lactating. Patients with hepatitis C or hepatitis delta co-infection were included.

Patients were randomized to receive either adefovir dipivoxil 10 mg daily (Gilead Sciences, East Melbourne, Victoria, Australia) or to continue their current prophylaxis with low-dose (800 IU per month) IM HBIG (CSL Bioplasma, Parkville, Victoria, Australia). Randomization was stratified by centre and was accomplished by sequential assignment of treatment numbers from lowest to highest, cross-referenced to a separately administered randomization spreadsheet. All patients continued to receive lamivudine 100 mg daily (GlaxoSmithKline Australia, Boronia, Victoria, Australia). Patients were reviewed (physical examination, physician assessment, urine pregnancy test for females of childbearing potential) at least monthly for the first three months and at least three-monthly thereafter. At each study visit, blood was taken for routine haematology and biochemistry, prothrombin time, hepatitis B surface antigen (HBsAg) and hepatitis B surface antibody (HBsAb). In addition, serum was stored at -70 degrees Celsius for
possible later reflex (if HBsAg positive) HBV DNA testing and resistance mutation sequencing.

The primary endpoint of the study was recurrence of HBV infection, defined as the reappearance of both HBsAg and HBV DNA in serum. The secondary endpoint of the study was to conduct an economic evaluation of the costs and outcomes of combination adefovir and lamivudine (ADV/LAM group) prophylaxis as compared to combination HBIG and lamivudine (HBIG/LAM group) prophylaxis. Analysis of data was performed when all patients reached 48 weeks post-randomization.

3.2.1 Baseline Antiviral Prophylaxis

The low-dose IM HBIG protocol used in all patients included in the study has been described previously (Angus, McCaughan et al. 2000). Prior to transplantation, patients with detectable serum HBV DNA (hybridization or PCR) received lamivudine 100 mg daily. Post-transplantation, patients received lamivudine 100 mg daily plus IM HBIG 800 IU daily for 1 week then monthly thereafter. At one centre (New South Wales), 400 IU HBIG was administered unless the trough HBsAb fell below 50 IU/L when the dose was increased to the 800 IU dose.

3.2.2 HBV assays

Viral markers (HBsAg, HBsAb, hepatitis B e antigen and antibody) were measured by standard commercial assays. HBV recurrence at the time of screening for entry into the study was excluded by seronegativity for both HBsAg and HBV DNA (using a standardized PCR assay: Roche COBAS AMPLICOR HBV monitor (Roche Molecular Systems, Branchburg, NJ, USA), lower level of detection 300 viral copies/mL). HBV DNA at latest reported follow-up was assessed by various commercial highly sensitive real time PCR assays (COBAS TaqMan HBV test (Roche Molecular Systems, Branchburg, NJ, USA), Abbott RealTime HBV (Abbott Molecular, Des Plaines, IL, USA), or Artus HBV LC PCR Kit (QIAGEN, Hilden, Germany) according to the local reference laboratory used by the transplant unit. The lower limit of detection (LLOD) for the three assays is quoted as 12 IU/mL, 15 IU/mL and 14 IU/mL, respectively.
Prior to study baseline, the three transplant centres used a variety of HBV DNA assays, depending on the year of transplantation and assay availability. The results of this testing were collected retrospectively in order to characterize the virological background of the groups. Results of HBV DNA testing were sought to determine the viral load at the commencement of antiviral therapy pre transplant, the time when HBV viral load became undetectable, and the HBV DNA level at transplant. Assays which reported results in picograms/mL or copies/mL were converted to IU/mL using the manufacturer’s recommended conversion multiple.

3.2.3 Statistical Methods

Continuous variables are expressed as median (range) unless specified otherwise. Categorical data are presented as number (percent). Cumulative patient survival and HBV recurrence-free survival were calculated using the Kaplan-Meier method. The Wilcoxon signed-rank test was used to detect change in serum creatinine over the course of the study. $P$ values <0.05 were considered statistically significant.
3.3 Results

Between September 2004 and July 2006, there were 147 patients who were potentially eligible for this study. These were post-liver transplant patients in Australia or New Zealand (without pre-transplant lamivudine resistance) who had received low dose HBIG and lamivudine prophylaxis. Of the 147, 24 patients were transplanted at sites not involved in this study, 20 patients had died (one with HBV recurrence), and another 4 patients were alive but had developed HBV recurrence and were started on adefovir as rescue therapy. This left 99 potential candidates who met inclusion criteria and were being treated at investigational sites. All eligible patients were approached unless they had known co-morbidity that precluded inclusion (8 patients with significant renal impairment, 2 patients with tumor, 3 patients who had been transplanted for fulminant hepatitis B, and 2 patients with HIV), or resided distant to the treating unit making the trial impractical (18 patients). This left 66 patients of whom 34 were recruited (16 in New Zealand, 9 in Victoria and 9 in New South Wales). The final obstacle to recruitment was failure to give consent since a significant proportion (32/65, or 48%) of eligible patients preferred to remain on their current therapy. Over the three sites, 16 patients were randomized to ADV/LAM and 18 to HBIG/LAM.

The demographic details of the 34 patients are included in Table 8. The median age (55.0 in the ADV/LAM group, 51.9 in the HBIG/LAM group), proportion of male patients (13/16 (81%) in ADV/LAM group, 15/18 (83%) in HBIG/LAM group) and ethnic background of the groups were similar. Median time since transplant until baseline was 4.4 years (range 1.1-6.3) in the ADV/LAM group and 4.6 years (1.3-7.2) in the HBIG/LAM group. Approximately half of all patients (10/16 (63%) in the ADV/LAM group vs. 8/18 (44%) in HBIG/LAM group) had a history of hepatocellular cancer prior to their transplant. One (6%) patient in the ADV/LAM group had hepatitis C co-infection (none in the HBIG/LAM group) and 2/18 (11%) patients in the HBIG/LAM group had hepatitis delta co-infection (none in the ADV/LAM group).
<table>
<thead>
<tr>
<th></th>
<th>ADV/LAM group (n=16)</th>
<th>HBIG/LAM group (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age</td>
<td>55 (26-67)</td>
<td>51.9 (37-69)</td>
</tr>
<tr>
<td>Years since transplant</td>
<td>4.4 (1.1-6.3)</td>
<td>4.6 (1.3-7.2)</td>
</tr>
<tr>
<td>Male gender</td>
<td>13 (81%)</td>
<td>15 (83%)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>6 (38%)</td>
<td>7 (39%)</td>
</tr>
<tr>
<td>European</td>
<td>5 (31%)</td>
<td>5 (28%)</td>
</tr>
<tr>
<td>South Pacific</td>
<td>5 (31%)</td>
<td>6 (33%)</td>
</tr>
<tr>
<td>Co-morbid liver disease aetiology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hepatoma</td>
<td>10 (63%)</td>
<td>8 (44%)</td>
</tr>
<tr>
<td>hepatitis C</td>
<td>1 (6%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>hepatitis delta</td>
<td>0 (0%)</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>alcohol</td>
<td>0 (0%)</td>
<td>1 (6%)</td>
</tr>
</tbody>
</table>

**Table 8: Patient demographics.**

Patients’ histories were reviewed to determine potential risk factors for post-transplant HBV recurrence and anti-viral breakthrough (Table 9). These data predate recruitment into the study and therefore a variety of HBV DNA assays were used, some of which were insensitive by modern standards. Nevertheless, the majority of patients had detectable HBV DNA at commencement of lamivudine therapy pre-transplantation, and the median HBV load prior to lamivudine was similar in both groups. The duration of lamivudine therapy prior to liver transplant was also similar in both groups. At transplant, 7/30 patients (4 unknown) had detectable HBV DNA (3 in the ADV/LAM group and 4 in the HBIG/LAM group).
### Table 9: Background virological risk factors for recurrence.

<table>
<thead>
<tr>
<th></th>
<th>ADV/LAM group (n=16)</th>
<th>HBIG/LAM group (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV DNA detectable prior to lamivudine (pre-transplant)</td>
<td>88%</td>
<td>70%</td>
</tr>
<tr>
<td>Median detectable HBV DNA prior to lamivudine (pre-transplant)</td>
<td>4.75 log&lt;sub&gt;10&lt;/sub&gt; IU/mL</td>
<td>5.5 log&lt;sub&gt;10&lt;/sub&gt; IU/mL</td>
</tr>
<tr>
<td>Time on lamivudine (pre-transplant)</td>
<td>4.1 months (1.2-13.5)</td>
<td>4.2 months (0.7-47.9)</td>
</tr>
<tr>
<td>Detectable HBV DNA at transplant</td>
<td>3 (two patients ≤ 4.4 log&lt;sub&gt;10&lt;/sub&gt; IU/mL, one 4.4-6.4 log&lt;sub&gt;10&lt;/sub&gt; IU/mL, 10 negative, 3 unknown)</td>
<td>4 (two patients ≤ 4.4 log&lt;sub&gt;10&lt;/sub&gt; IU/mL, one 4.4-6.4 log&lt;sub&gt;10&lt;/sub&gt; IU/mL, and one ≥ 6.4 log&lt;sub&gt;10&lt;/sub&gt; IU/mL, 13 negative, 1 unknown)</td>
</tr>
</tbody>
</table>

#### 3.3.1 Patient survival & withdrawal from trial.

All patients in both groups were alive at the time of manuscript preparation. Median follow up was 21.1 months (9.4-35.9) in the ADV/LAM group and 21.8 months (13.5-35.6) in the HBIG/LAM group. At 3 months following randomization, one patient in the ADV/LAM group withdrew consent from the study and is not considered in further results. This patient was switched back to HBIG/LAM and has not developed recurrence with a further 28 months of follow up.

#### 3.3.2 HBsAb levels

The median trough HBsAb level in the HBIG/LAM group was approximately 50IU/L throughout the study (see Figure B). As expected, HBsAb levels fell rapidly
in the ADV/LAM group, and 6 months after HBIG cessation no patient was found to have a HBsAb level > 10IU/L.

\[ \text{Figure 8: Trough HBsAb titre vs. time.} \]

### 3.3.3 Recurrent Hepatitis B

There were no cases of recurrence (defined as detectable HBsAg and HBV DNA by highly sensitive real time PCR assay) in either arm of the study. One patient in the ADV/LAM group had detectable HBsAg (titre 0.05 IU/mL) at 5 months however HBV DNA was undetectable by PCR (Artus RealArt, LLOD 14 IU/mL) at the time and when tested repeatedly over the following 24 months. The patient displayed no clinical or biochemical evidence of hepatitis at any time.

### 3.3.4 Safety & Renal Function

Median creatinine was unchanged over the course of the study in both groups. In one patient in the ADV/LAM group, serum creatinine rose significantly following inclusion in the study necessitating adefovir dose reduction and finally withdrawal from the study. This patient had pre-existing diabetic and hypertensive nephropathy and in the two years prior to baseline serum creatinine had fluctuated between 130μmol/L and 180μmol/L. Baseline creatinine at study entry was 150μmol/L. Over the next 15 months, adefovir was dosed according to the manufacturers recommendation. At 15 months serum creatinine was persistently
above 230 µmol/L. Adefovir therapy was ceased and treatment switched back to HBIG/LAM. At that time there was no evidence of recurrence and the patient has remained HBsAg and HBV DNA negative since. Since cessation or adefovir serum creatinine has not improved, fluctuating between 210 µmol/L and 250 µmol/L.

<table>
<thead>
<tr>
<th></th>
<th>positive HBsAg</th>
<th>detectable HBV DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADV/LAM group</td>
<td>1/15 (6%)</td>
<td>0/18 (0%)</td>
</tr>
<tr>
<td>HBIG/LAM group</td>
<td>0/15 (0%)</td>
<td>0/18 (0%)</td>
</tr>
</tbody>
</table>

**Table 10: HBV recurrence at latest follow-up.**

<table>
<thead>
<tr>
<th>Creatinine (µmol/L)</th>
<th>Start median</th>
<th>End median</th>
<th>Change over study</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADV/LAM group (n=15)</td>
<td>107 µmol/L</td>
<td>108 µmol/L</td>
<td>+1 µmol/L</td>
</tr>
<tr>
<td>HBIG/LAM group (n=18)</td>
<td>109 µmol/L</td>
<td>108 µmol/L</td>
<td>-1 µmol/L</td>
</tr>
</tbody>
</table>

**Table 11: Renal function.**

There were no other significant adverse advents considered related to therapy in either arm of the study.
3.3.5 Costs of therapy

Cost elements (Table 12) were obtained from the largest single centre (the New Zealand Liver Transplant Unit) and converted into $US (exchange rate: $US 1 = $NZ 1.31). Total costs include drug (adefovir dipivoxil 10mg daily and lamivudine 100mg daily vs. HBIG 800 IU monthly and lamivudine 100mg daily), attendance (quarterly vs. monthly for IM HBIG injections), and minimum mandatory serological testing (quarterly HBsAg vs. monthly HBsAb testing). Societal costs (patient time, travel, and loss of income) are not included. Equal efficacy in prophylaxis was assumed.

The total cost/year in the ADV/LAM group was $8,290 and the total cost/year in the very low dose IM HBIG/LAM group was $13,718 yielding a yearly saving of $5,428 in favour of combination ADV/LAM prophylaxis.

<table>
<thead>
<tr>
<th>Cost element</th>
<th>Cost per year</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBIG</td>
<td>$10,410</td>
<td>New Zealand Pharmaceutical Schedule</td>
</tr>
<tr>
<td>Lamivudine</td>
<td>$1426</td>
<td>New Zealand Pharmaceutical Schedule</td>
</tr>
<tr>
<td>Adefovir</td>
<td>$6236</td>
<td>New Zealand Pharmaceutical Schedule</td>
</tr>
<tr>
<td>Visits &amp; serology (HBIG/LAM group)</td>
<td>$1882</td>
<td>Auckland Hospital Schedule</td>
</tr>
<tr>
<td>Visits &amp; serology (ADV/LAM group)</td>
<td>$627</td>
<td>Auckland Hospital Schedule</td>
</tr>
</tbody>
</table>

Table 12: Cost elements.
3.4 Discussion

A number of strategies have been proposed to avoid the costs and inconvenience associated with long-term high dose IV HBIG administration for post liver transplant hepatitis B prophylaxis. In Australia and New Zealand, the lack of availability of an intravenous HBIG preparation has lead to the adoption of a low dose IM HBIG and lamivudine regimen for prophylaxis against HBV recurrence (McCaughan, Spencer et al. 1999; Angus, McCaughan et al. 2000). In 147 patients transplanted over seven years the rate of HBV recurrence was 4% (Gane, Angus et al. 2007), similar to rates achieved with high-dose IV HBIG based regimes, regardless of pre-transplant HBV DNA status. This low dose protocol reduces the direct expenses of HBIG, but it does not avoid the cost and inconvenience associated with the long-term administration of HBIG. An alternative strategy is to simply cease IV HBIG at some point post transplant whilst continuing lamivudine. However, even in patients who have no detectable HBV DNA at transplant (and are therefore considered at lower risk of recurrence), HBIG cessation at various time points post-transplant has been found to be associated with a significant risk of recurrence (up to 13% at 5 years) (Buti, Mas et al. 2005). Post-transplant HBV vaccination has been used in some studies with the aim of achieving a durable endogenous surface antibody response, thereby eliminating the need for continued exogenous HBIG. Although very good results have been reported in one study (Bienzle, Gunther et al. 2003), other investigators have been unable to achieve similar success (Lo, Liu et al. 2005; Rosenau, Hooman et al. 2007), and this approach, although attractive, remains experimental.

In this current study, long-term prophylaxis with low dose IM HBIG and lamivudine was replaced with adefovir dipivoxil and lamivudine in patients who were at least 12 months post-transplant without evidence of HBV recurrence or lamivudine resistance. Adefovir was chosen for this study since it has been shown
to be generally safe and well tolerated in liver transplant recipients (Perrillo, Hann et al. 2004; Schiff, Lai et al. 2007). Adefovir has been shown to be effective both in improving hepatitis and indices of liver function in patients with lamivudine resistance pre-transplant and in the setting of post-transplant hepatitis B recurrence. Furthermore, lamivudine and adefovir represent two different classes of anti-HBV agents (L-nucleoside and acyclic phosphonate) with different mechanisms of action and without cross-resistance and this combination greatly reduces the risk of the development of subsequent adefovir resistance in non-transplant patients (Lampertico, Vigano et al. 2007; van der Poorten, Prakoso et al. 2007).

The combination of adefovir and lamivudine was effective in preventing recurrence following the cessation of HBIG. Importantly, the majority of patients in both arms of the study had detectable HBV DNA prior to lamivudine therapy pre-transplant with a moderate viral load, and a significant minority in both groups still had detectable HBV DNA at time of transplant (table 2). Thus, most patients were at high risk of the development of HBV recurrence in the absence of effective prophylaxis. Although median follow-up in the patients who have been switched is 21 months and a majority have been followed for more than two years, it is possible that with more prolonged follow-up some patients may experience viral breakthrough due to the development of combined adefovir and lamivudine resistance. This is unlikely since none of the patients had pre-existing lamivudine resistance and resistance to both adefovir and lamivudine is very rare when the two are used in combination (Lampertico, Vigano et al. 2007; van der Poorten, Prakoso et al. 2007).

It was of considerable interest that one patient in the ADV/LAM group developed detectable HBsAg (at a low titre of 0.05 IU/mL) at five months but has remained persistently undetectable HBV DNA by highly sensitive PCR assay (LLOD 14 IU/mL) over a further 20 months of follow up. The likely explanation for this was that HBsAg was being produced at a low level prior to inclusion in the study and that this was unmasked as HBsAb titres fell following the switch to adefovir/lamivudine (see Figure 8). This finding is in keeping with studies which
have shown that the majority of patients who have no evidence of HBV reinfection whilst receiving long-term HBIG have detectable HBV DNA sequences within the liver (Roche, Feray et al. 2004). Importantly the combination of adefovir and lamivudine has prevented the development of measurable HBV replication in this patient.

The use of combination adefovir/lamivudine for post-transplant prophylaxis resulted in significant cost savings compared to standard low dose HBIG/lamivudine therapy. The cost of both treatments was very much less than that of continuing high dose IV HBIG, which is approximately $50,000 USD per year (Dan, Wai et al. 2006). Furthermore this regime is significantly less burdensome to patients, allowing three-monthly rather than monthly clinic visits and obviating the need for parenteral administration. Although the regime was well tolerated, the one patient who developed progressive renal impairment during the study illustrates the need to monitor renal function. It should be noted that this patient had significant renal impairment pre transplant that was attributed to diabetic nephropathy and also had difficult to control hypertension. It is likely that the deterioration in renal function during the course of the study was not primarily due to adefovir use since renal function has not improved after the drug was ceased.

It is possible that combination adefovir/lamivudine therapy could also provide adequate HBV prophylaxis in patients with less than 12 months of initial post-transplant HBIG/lamivudine therapy (Nath, Kalis et al. 2006). Furthermore, primary prophylaxis with combination lamivudine and adefovir from the time of listing may be effective and this is explored in the next chapter of this thesis. It is likely that other antiviral combinations could provide similar protection to adefovir/lamivudine, the underlying principle in choosing which combination to use in future studies being to maximize the virological hurdle for resistance whilst minimising therapeutic costs and side effects.

In conclusion, in patients who are at least 12 months post-transplant, the switch to combination adefovir/lamivudine provides effective prophylaxis against recurrence, equivalent to that provided by ongoing HBIG/lamivudine therapy.
Furthermore, combination adefovir/lamivudine therapy is less expensive and is less burdensome to patients. These findings support the use of combination adefovir and lamivudine in preference to long-term HBIG therapy in patients transplanted for HBV related liver disease.
Chapter 4. Avoiding hepatitis B

prophylaxis with HBIG: a prospective study of combination lamivudine and adefovir initiated at wait-listing for HBsAg positive liver transplant candidates.
4.1 Introduction

In Chapter three I have shown that, in patients who are at least 12 months post-liver transplant for HBsAg positive disease and receiving maintenance low-dose IM HBIG and lamivudine, the switch to combination adefovir/lamivudine provides effective prophylaxis against recurrence (equivalent to that provided by ongoing HBIG/lamivudine therapy). Such management significantly reduces the cost and inconvenience incurred with ongoing HBIG-containing prophylaxis (Angus, Patterson et al. 2008). The natural question that follows from this is: to what extent can we reduce HBIG therapy from post-transplant prophylaxis regimes without compromising outcomes?

Post-transplant HBV prophylaxis without HBIG was studied upon the availability of lamivudine. Unfortunately, lamivudine mono-prophylaxis was disappointing, with 40-50% of patients developing recurrence at three years (Mutimer, Dusheiko et al. 2000; Perrillo, Wright et al. 2001). Cessation of HBIG after a defined period of combination HBIG/lamivudine prophylaxis (including prior to 12 months) has also been explored. In the largest prospective study, 20 patients were treated with between one month (in 14 patients) and 18 months (in six patients) of HBIG/lamivudine before switching to lamivudine alone (Buti, Mas et al. 2003). After five years, recurrence was seen in three patients (15%) although this result is clouded by significant non-compliance to therapy (Buti, Mas et al. 2007). Clearly lamivudine alone (even after a period of combination therapy) is not adequate to satisfactorily prevent HBV recurrence, and this has led some to believe HBIG is of cardinal importance in the prophylaxis of HBV recurrence.

The putative modes of action of HBIG in the prevention of post-transplant recurrence were thought to be both clearance of HBsAg encapsulated particles from serum and blockade of viral entry (mediated by a HBsAg receptor) into
allograft hepatocytes (Shouval and Samuel 2000). This theory is now recognised as false, as the majority of patients successfully managed with combination high dose IV HBig and lamivudine still have HBV detectable in either liver or serum (by sensitive PCR-based assays) (Roche, Feray et al. 2003; Hussain, Soldevila-Pico et al. 2007). Clearly, successful prophylaxis of HBV recurrence with HBig does not preclude re-infection, rather the suppression of low-level infection from progressing to overt disease recurrence. The availability of new antiviral agents (including oral antiviral combination therapy) may permit the same suppression without the requirement for HBig.

As discussed and shown in Chapter three, combination lamivudine and adefovir therapy is safe in liver transplant recipients. The risk of antiviral resistance to both drugs is small when used in combination even after the occurrence of lamivudine resistance (Perrillo, Hann et al. 2004; Schiff, Lai et al. 2007). This chapter describes a prospective study of combination adefovir and lamivudine initiated at waitlisting for liver transplant and continued as primary post-transplant as prophylaxis against HBV recurrence, with HBig therapy limited to the immediate peri-transplant period.
4.2 Methods

This was a prospective, multi-centre trial conducted at the New Zealand (Auckland City Hospital, New Zealand), Victorian (Austin Health, Melbourne, Australia) and New South Wales (Royal Prince Alfred Hospital, Sydney, Australia) liver transplant units between August 2003 and March 2007. The appropriate institutional ethics review committees approved the trial prior to commencement and all subjects gave written informed consent prior to entry into the study.

All hepatitis B surface antigen (HBsAg) positive adults listed for liver transplantation who had not received antiviral therapy and those who had been commenced on lamivudine prior to referral due to detectable HBVDNA were included. Patients receiving lamivudine prior to the study were excluded if they had genotypic mutations known to convey lamivudine resistance (rtM204I/V, rtL180M, rtV173L, etc.). Patients were also excluded if they had recent exposure (less than three months) to anti-HBV agents other than lamivudine, fulminant hepatic failure, a serum creatinine of ≥180μmol/L or if they were HIV seropositive (or other significant co-morbidity) pregnant or lactating. Patients with hepatitis C or hepatitis delta co-infection were not excluded.

All patients received lamivudine 100 mg daily (GlaxoSmithKline Australia, Boronia, Victoria, Australia) and adefovir dipivoxil 10 mg daily (Gilead Sciences, East Melbourne, Victoria, Australia). Dosage was adjusted to renal function according to the manufacturers’ recommendation. Patients were reviewed (physical examination, physician assessment, urine pregnancy test for females of childbearing potential) at least monthly pre-transplant and at least three monthly post-transplant. At each study visit, blood was taken for routine haematology and biochemistry, HBsAg, antibody to HBsAg (HBsAb or anti-HBs). At pre-transplant study visits, blood was additionally tested for HBV DNA by PCR (Roche COBAS AMPLICOR HBV MONITOR assay or sensitive real time PCR assay) and prothrombin time (which enabled calculation of a MELD score). At post-transplant study visits,
an additional 2mL of serum was stored at -70 degrees Celsius to allow for later reflex (if HBsAg positive) HBV DNA testing and resistance mutation sequencing. At study completion, all patients had real time HBV PCR assays performed regardless of HBsAg status or time post transplant.

At transplant, in addition to continuing lamivudine and adefovir therapy, patients received 800 IU intramuscular HBIG (CSL Bioplasma, Parkville, Victoria, Australia) during the anhepatic phase and daily for seven days post-transplant (total 6400 IU HBIG administered over 7 days) according to our previous protocol (21). This administration of HBIG was not primarily for prophylactic benefit but rather as a precaution in the event that post-transplant renal impairment might preclude ongoing adefovir therapy. Patients did not receive any subsequent HBIG.

The primary endpoint of the study was recurrence of HBV infection post-transplant, defined as the reappearance of both HBsAg and HBV DNA in serum. The secondary endpoints were outcomes of patients prior to transplantation (degree and durability of HBV suppression, death or delisting prior to transplant), the occurrence of adverse events and change in renal function.

### 4.2.1 HBV assays

Viral markers (HBsAg, HBsAb, hepatitis B e antigen and antibody) were measured by standard commercial assays. HBV DNA at screening for entry into the study was by the Roche COBAS AMPLICOR HBV monitor (lower level of detection approximately 55 IU/mL). HBV DNA at latest reported follow-up was assessed by various commercial highly sensitive real time PCR assays (COBAS TaqMan HBV test (Roche Molecular Systems, Branchburg, NJ, USA), Abbott RealTime HBV (Abbott Molecular, Des Plaines, IL, USA), or Artus HBV LC PCR Kit (QIAGEN, Hilden, Germany) according to the local reference laboratory used by the transplant unit. The lower limit of detection (LLOD) for the three assays is quoted as 12 IU/mL, 15 IU/mL and 14 IU/mL, respectively.

Prior to study baseline the three transplant centres used a variety of HBV DNA assays, depending on the year of transplantation and assay availability. These results of this testing were collected retrospectively in order to characterize the
virological background of the groups, particularly the viral load at the commencement of antiviral therapy pre-transplant. Results reported in picograms/mL or copies/mL were converted to IU/mL using the manufacturer’s recommended conversion.

4.2.2 Statistical Methods

Continuous variables are expressed as median (range) unless specified otherwise. Categorical data are presented as number (percent) Cumulative patient survival and HBV recurrence-free survival were calculated using the Kaplan-Meier method. The Wilcoxon signed-rank test was used to detect change in serum creatinine over the course of the study. $P$ values $<0.05$ were considered statistically significant.
### 4.3 Results

Between August 2003 and March 2007, 26 patients HBsAg positive patients who were potentially eligible for this study at the time of waitlisting for liver transplant were recruited to the study. Sixteen were recruited in New Zealand, five in Victoria and five in New South Wales. The demographic details of the 26 patients are included in Table 13: Baseline characteristics. The median age was 52 years (range 19.1 – 65.4) and 19/26 (73%) were male. Ethnic background was Asian (42%), Pacific Islander (31%) and Caucasian (27%). Over half of all patients (62%) had a history of hepatocellular cancer and one patient had hepatitis delta co-infection. No patients were HCV co-infected. The median MELD score unadjusted for hepatoma at baseline was 14.3 (range 6-34).

<table>
<thead>
<tr>
<th></th>
<th>n=26</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median age (range)</strong></td>
<td>52 (19.1-65.4)</td>
</tr>
<tr>
<td><strong>Male gender</strong></td>
<td>19/26 (73%)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
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<tr>
<td></td>
<td>Asian</td>
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<td></td>
<td>European</td>
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<td>South Pacific</td>
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<td><strong>Co-morbid liver disease</strong></td>
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<tr>
<td></td>
<td>HCC</td>
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<td></td>
<td>HDV</td>
</tr>
<tr>
<td><strong>Median MELD (range)</strong></td>
<td>14.3 (6-34)</td>
</tr>
</tbody>
</table>

**Table 13: Baseline characteristics.**

HCC: hepatocellular cancer. HDV: hepatitis delta virus. MELD: model for end-stage liver disease.
The virological background of patients recruited into the study is detailed in Table 14. Prior to baseline, 12/26 patients (46%) were receiving lamivudine, although the duration of therapy was short: (median 1.9 months, range 0.6-6.9). All 12 patients had detectable HBV DNA prior to lamivudine and the median viral load was $\log_{10} 4.0$ IU/mL (range 2.3-7.5). At baseline, 21/26 patients (81%) had detectable HBV DNA by PCR (LLOD 55 IU/mL) and 41% were HBeAg positive. The five patients who were HBV DNA negative had commenced lamivudine therapy prior to entering the study. Of those with detectable HBV DNA at baseline, the median viral load was $\log_{10} 3.3$ IU/mL (range 1.8-8).

<table>
<thead>
<tr>
<th></th>
<th>n=26</th>
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<tbody>
<tr>
<td>Lamivudine prior to study</td>
<td>12/26 (46%)</td>
</tr>
<tr>
<td>Duration of prior LAM (range)</td>
<td>1.9 months (0.6-6.9)</td>
</tr>
<tr>
<td>Median HBV DNA* prior to LAM (range)</td>
<td>$4.0 \log_{10}$ IU/mL (2.3-7.5)</td>
</tr>
<tr>
<td>Detectable HBV DNA† at baseline</td>
<td>21/26 (81%)</td>
</tr>
<tr>
<td>Median detectable HBV DNA at baseline</td>
<td>$3.3 \log_{10}$ IU/mL (1.8-8.0)</td>
</tr>
<tr>
<td>Baseline eAg status</td>
<td>11/26 (42%)</td>
</tr>
</tbody>
</table>

**Table 14: Virological risk factors for recurrence.**

LAM: lamivudine. HBV: hepatitis B virus. * variety of assays (prior to study). † standardized assay: Roche COBAS AMPLICOR HBV, LLOD ~55 IU/mL.

**4.3.1 Pre-transplant patient survival & withdrawal.**

Whilst waiting for liver transplant, two patients were delisted for hepatocellular cancer progression beyond transplant criteria (both within three months of
baseline), two patients were delisted for improvement (MELD scores improving from 23 to 13 in one patient and unchanged at 8 in the other) and one patient died of cryptococcal sepsis, 2 months after study entry. At the time of data analysis, two patients were yet to be transplanted. In the remaining 19 patients who survived to transplantation, median MELD score at review immediately pre-transplant was 17 (range 6-34).

4.3.2 Post-transplant survival and withdrawal.

Nineteen patients underwent liver transplantation. The median waiting time was 3.6 months (range 0-17 months). At transplant, 9/18 patients (one patient did not have HBV DNA performed at transplant) had detectable HBV DNA with a median viral load in those patients of 2.6 log10 IU/mL (range 1.4-4.7). In those who became HBV DNA negative, the mean time to an undetectable (LLLOD 55 IU/mL) HBV DNA was 3.9 months (range 0.9-7.8 months). At transplant 20% of patients were HBeAg positive.

All nine patients who had quantifiable HBV viral load at time of transplant became undetectable by real time PCR assay during follow-up; this occurred at a median of 1.5 (range 0.8 – 3.2) months post-transplant. The one patient who did not have a HBV viral load at transplant had a low viral load (416 IU/mL) at baseline 3 months prior to transplant and was HBV DNA negative at two months post-transplant. One patient with HBV load of 140 IU/mL at transplant remained HBsAg positive until three months post-transplant at which point the HBV load was <12 IU/mL. Thereafter HBsAg was undetectable.

All transplanted patients were alive and continued to be monitored on the study at the time of manuscript preparation. Median follow up post-transplant was 11.7 (range 1-40) months.

4.3.3 Recurrent hepatitis B

There were no cases of HBV recurrence (defined as detectable HBsAg and HBV DNA in serum). HBsAg was assayed at every visit post-transplant and no patients became newly HBsAg positive (one patient remained HBsAg detectable for the
initial three months post-transplant as discussed). At study completion all patients
has both HBsAg and HBV DNA measured. All remained HBsAg negative and HBV
DNA undetectable by real time HBV PCR assay (LLOD not greater than 15 IU/mL).

4.3.4 Safety & renal function

Median creatinine increased by 33 µmol/L over the course of the study, from 81
µmol/L at baseline to 114 µmol/L at last follow-up. Median creatinine at time of
transplant was 114 µmo/L. Median total duration of combination adefovir
dipivoxil and lamivudine exposure was 27 months (range 13-57 months). There
were no patients for whom adefovir or lamivudine therapy had to be ceased, nor
did any patient require alteration of adefovir or lamivudine dose or dosage
interval. There were no serious adverse advents reported which were considered
to be related to study therapy.
4.4 Discussion

This current study demonstrates that the combination of adefovir and lamivudine is highly effective in preventing post-liver transplant hepatitis B recurrence, obviating the need for ongoing prolonged HBIG therapy. After a median follow-up of almost two years, no patient developed recurrence and there were no significant adverse events. Importantly, the vast majority of patients in this study had detectable HBV DNA prior to antiviral therapy and a significant minority had ongoing detectable HBV DNA at time of transplant. Thus, in the absence of effective prophylaxis, these patients were at high risk of HBV recurrence. As expected, in some patients treatment with this combination resulted in clinical improvement pre-transplant enabling them to be removed from the waiting list.

Adefovir was chosen as the second antiviral for this study as it has been shown to be generally safe and well tolerated in liver transplant recipients (Perrillo, Hann et al. 2004; Schiff, Lai et al. 2007). Furthermore, it is effective both in improving hepatitis and indices of liver function in patients with lamivudine resistance pre-transplant and in the setting of post-transplant hepatitis B recurrence (Perrillo, Hann et al. 2004). The study outlined in Chapter three showed that adefovir is safe and effective when substituted for HBIG at more than 12 months post transplant in patients receiving long-term HBIG lamivudine therapy. Importantly, to qualify for the current study, patients exposed to lamivudine had to be free from genotypic lamivudine resistance, because of concerns that the addition of adefovir might not provide sufficient suppression of viral replication pre-transplant in patients whose viral replication was not controlled by lamivudine.

As discussed in the last chapter, it is possible that with more prolonged follow-up patients in our study may experience viral breakthrough due to the development of combined adefovir and lamivudine resistance. We believe this is unlikely as ongoing combination adefovir and lamivudine therapy instituted at onset of lamivudine resistance greatly reduces the risk of the development of subsequent
adefovir resistance (Lampertico, Vigano et al. 2007; van der Poorten, Prakoso et al. 2007). This has led to the assumption that combination lamivudine and adefovir therapy in nucleo(s) tide naïve patients might also be particularly effective at avoiding resistance and breakthrough. In a recent study, de novo combination therapy had modestly superior virological and biochemical outcomes over lamivudine mono-therapy, and was associated both with significantly less lamivudine resistance (15% vs. 43%) and virological breakthrough (Sung, Lai et al. 2008). However, considering the efficacy of combination adefovir and lamivudine in lamivudine resistant patients, the absolute rate of breakthrough in the combination group (19%) was surprising. Reassuringly (with particular regard to the current study protocol) in that study no cases of combination genotypic resistance to both adefovir and lamivudine occurred with 2 years of therapy.

Several small short-term retrospective studies of combination adefovir/lamivudine therapy for post-liver transplant HBV prophylaxis have provided promising results that support the findings of the current study. In a study of 16 HBsAg positive transplant candidates, adefovir was added to lamivudine pre-transplant at the onset of lamivudine resistance (Lo, Liu et al. 2005). Eight of these patients were later transplanted without HBIG. At a median follow up of 11 months, all remained HBV DNA negative, although two were HBsAg positive. Another retrospective study reported on 14 patients who commenced long-term adefovir and lamivudine at the time of transplant, with HBIG only given for the first week post-transplant (Nath, Kalis et al. 2006). Seven of these patients received pre-transplant lamivudine and one had documented lamivudine resistance. One patient failed to clear HBsAg and remained HBV DNA positive, whereas the remaining patients were both HBsAg and HBV DNA negative at a median of 14 months post-transplant. Finally, although not designed to specifically examine the effectiveness of adefovir/lamivudine combination therapy, a large prospective study of compassionate-use adefovir in 467 waitlisted or post-transplant patients with lamivudine-resistance included 61 patients who underwent transplant whilst on adefovir, of whom 23 did not receive HBIG. The rate of HBV recurrence after a median of 36 weeks post-transplant was not influenced by the use of HBIG (12% with HBIG vs. 13% without), however the
short follow-up and a lack of data on some patients (especially any concurrent lamivudine therapy) limit the conclusions that may be drawn.

It is likely that other potent antiviral agents or antiviral combinations could also provide effective protection without the use of HBIG. Based on the their high potency and very low resistance rates the newer anti-HBV drugs antiviral agents such entecavir or tenofovir are attractive candidates for future studies with the underlying principle being to maximize the virological hurdle for resistance whilst minimising therapeutic costs and side effects.

In Chapter three it was found that the use of combination adebovir/lamivudine for post-transplant prophylaxis resulted in significant cost savings compared to standard low dose HBIG/lamivudine therapy: $8290 vs. $13,718 per year (Angus, Patterson et al. 2008) and was very much less than that of continuing high dose IV HBIG, which is approximately $50,000 USD per year (Dan, Wai et al. 2006). Furthermore this regime is significantly less burdensome to patients, allowing three-monthly rather than monthly clinic visits and obviating the requirement for parenteral therapy. Although the regime was well tolerated, adebovir dipivoxil can lead to deterioration in renal function and this must be monitored. However, the change in renal function observed over the course of this study (median +33 μmol/L over median 11.7 months post-transplant) was not greater than might expect in patients receiving calcineurin inhibitor therapy after liver transplantation (Ojo, Held et al. 2003) and this suggests that to date any possible additive renal toxicity from adebovir has been minor.

In conclusion, combination adebovir/lamivudine suppresses HBV replication pre-transplant and provides effective medium term prophylaxis against post-transplant recurrence. The combination was well tolerated and there was little evidence to suggest a significant additive renal toxicity from the drug in patients receiving calcineurin antagonist therapy. These findings provide further evidence that this combination (and likely other potent antiviral drugs or combinations) can provide durable, safe and effective HBV prophylaxis without long-term HBIG resulting in a major reduction in the cost of managing HBsAg positive liver transplant recipients.
Chapter 5: Summary and Conclusions
Hepatitis B is a major global health problem with an estimated 2 billion people having been infected and 350-400 million people suffering from chronic infection. The hepatitis B virus is a hepatotropic DNA virus that replicates via an RNA intermediary and this explains the relatively high mutation rate observed in comparison to other DNA viruses. Hepatitis B has a worldwide distribution but is endemic in countries where neonatal or infantile transmission is common as this is frequently followed by chronic infection. Liver injury in patients with chronic hepatitis B disease is a consequence of attempted immune clearance, and in many patients this results in progressive liver fibrosis and ultimately cirrhosis or hepatocellular cancer. As a consequence, HBV is the 10th leading cause of death worldwide and the complication hepatocellular cancer is the 3rd most common cause of cancer death worldwide.

The management of chronic hepatitis B has been transformed over the last 2 decades by the development of a range of effective therapies. These are primarily oral nucleos(t)ide agents, parenteral interferon-alpha therapy and, in the setting of liver transplantation, hepatitis B immune globulin. All these treatments may lead to durable suppression of viral replication however in most patients complete clearance of infection is not a realistic aim. As a result long-term treatment long-term is frequently needed and, as with chronic therapy in other infectious diseases, this may be compromised by the development of drug resistance. Drug resistance both reduces the efficacy of therapy and limits future treatment options.

A key goal in the therapy of chronic HBV with oral anti-HBV agents has therefore been to develop effective strategies for the prevention and treatment of drug resistance. This has been of particular importance in the setting of previous drug resistance or poor response to initial therapy and in prophylaxis of HBV recurrence post-liver transplantation, where failure of prophylaxis due to antiviral resistance may lead to rapidly progressive graft injury and death. This thesis describes the use of novel antiviral treatment strategies in these two settings.
Chapter two describes a novel study with a new antiviral, tenofovir disoproxil fumarate, which examined its efficacy and safety in managing patients who had previously failed both lamivudine and adefovir dipivoxil and had significant ongoing viral replication. Due to the chronology of the introduction of these drugs in Australia and their antiviral characteristics there is now a significant population of chronic HBV patients in with this problem. Chronic lamivudine therapy is frequently complicated by lamivudine resistance (65% at 5 years) and adefovir, though associated with less antiviral resistance, is less potent than lamivudine and furthermore, some patients show primary non-response to this drug. Adefovir rescue therapy involving a switch from lamivudine at the onset of lamivudine failure is therefore often not successful and such patients previously had no ideal therapeutic option.

Tenofovir is structurally related to adefovir, however, because it is less nephrotoxic it can be given in much greater dosage (300mg vs. 10mg) and thus in treatment naïve patients at standard doses tenofovir has significantly greater antiviral activity than adefovir. As discussed in Chapter two, a prospective open label study was conducted of tenofovir treatment in 60 such patients recruited from 7 tertiary hospitals in Victoria and New South Wales. All enrolled patients had both failed lamivudine in the past and either failed or had a suboptimal response to adefovir over at least 24 weeks immediately preceding study baseline. This was a heavily pre-treated cohort of patients, with a median 37 months of previous lamivudine exposure and 22 months of adefovir exposure. Mutations conferring resistance to LAM or ADV were present in 20/60 (33%) and 17/60 (28%) patients, respectively.

With the exception of one patient who discontinued tenofovir after 10 days due to the development of a rash, all patients have been followed for a minimum of 96 weeks on study. No other adverse events considered related to tenofovir occurred nor was there any significant change in renal function over the duration of the study. The median baseline viral load was $5.33 \log_{10} \text{IU/mL}$ (range 2.81-8.04). The median change in HBV DNA from baseline to week 12 was the primary endpoint and there was a significant response at 12 weeks in all patients with a median fall
in HBVDNA of -2.86 log\textsubscript{10} IU/mL. Median HBV DNA continued to fall throughout the study, and at last follow-up at 96 weeks had fallen by -4.03 log\textsubscript{10} IU/mL. The median change in viral load from baseline was unrelated to baseline HBeAg status, ongoing lamivudine therapy or the presence of genotypic resistance mutations. At 48 weeks 27/59 (46%) patients achieved a HBV DNA <15 IU/mL and at 96 weeks 38/59 (64%) patients achieved the same endpoint. The proportion of patients who achieved a viral load <15 IU/mL was not significantly related to ongoing lamivudine therapy or the presence of genotypic resistance mutations, however there was a transient association with HBeAg positive status (p<0.05 to 48 weeks on study, p=ns at >48 weeks). There was no HBeAg seroconversion or HBsAg seroconversion observed. Four patients have acquired sequence mutations on study (two with rtA181T, one with rtN236T and one with both rtA181T and rtN236T) but no novel resistance mutations have been detected.

Tenofovir therefore shows significant antiviral activity with the majority of these difficult to treat patients achieving undetectable viral loads (by sensitive PCR-based assay) after 2 years of therapy. The performance of tenofovir in this cohort is both inferior to that observed in naïve patients as well as inferior to the published experience of tenofovir in difficult to treat (antiviral resistant) patients. The probable explanation is that this cohort was more heavily pre-treated and had a higher rate of genotypic resistance. However, the only remaining alternative for patients in this situation is entecavir which, although a potent and possessing a favourable resistance profile in naïve patients, is severely compromised in the setting of established lamivudine resistance (genotypic resistance in 15% at 2 years and 51% at 5 years). The results of this thesis show that tenofovir should be the preferred choice of antiviral for patients with previous lamivudine and adefovir resistance.

Although a significant relationship between the presence of viral mutations conferring adefovir resistance at baseline and persistent viral replication >15 IU/mL was not observed (p=0.085 at 96 weeks in the case of baseline rtN236T), it is striking that five of seven (71%) patients with baseline rtN236T substitution have persistent replication at 96 weeks. Furthermore, three of these five patients
have had no significant fall in HBV DNA since 36 weeks. This observation, combined with the selection for adefovir resistance mutations during the study suggests that such a relationship may eventuate. This is not unexpected, as the adefovir resistance mutations rtA181T and rtN236T show a degree of cross-resistance to tenofovir *in vitro* and a degree of clinical cross-resistance has been suggested in a recent retrospective study.

It should be anticipated that novel mutations conferring tenofovir genotypic resistance will be observed eventually. Virological principles would indicate that this is more likely to occur in the setting of substantial antiviral exposure and an incomplete response to therapy. It will therefore be of major interest to follow the cohort of patients described in Chapter two and especially those who have remained HBV DNA positive or have adefovir resistance mutants detectable in serum. An extension to this study is therefore currently being undertaken. In addition, the value of combination therapy has not been resolved and further randomized studies are needed.

Post-transplant prophylaxis of hepatitis B recurrence has evolved substantially over the last 20 years. Prior to the availability of HBIG, liver transplantation for hepatitis B was contraindicated due to the high rate of hepatitis B recurrence and this usually lead to loss of the allograft and patient death. With the use of HBIG alone, and later HBIG in combination with lamivudine, recurrence rates fell to <5% at five years and this has resulted in liver transplantation for hepatitis B being widely performed with much more favourable outcomes. Hepatitis B viral load at transplant and viral load prior to the institution of pre-transplant antiviral therapy is the most important risk factor for post-transplant recurrence.

Unfortunately long-term HBIG therapy is costly (~$US 100,000 in the first year and $US 50,000 yearly thereafter) and inconvenient to patients (requiring regular parenteral administration). This thesis prospectively examined two strategies to reduce the requirement for HBIG, both of which entailed the use of combination lamivudine and adefovir. This combination has proven to be safe and effective in other clinical contexts and there is considerable experience with the use of adefovir monotherapy in both end-stage chronic liver disease and post-liver
transplant management. In addition, the combination of lamivudine and adefovir when instituted at or before lamivudine failure is associated with a low rate of combination resistance and there is limited potential for cross-resistance between the two drugs. Chapter four describes substitution of adefovir dipivoxil for HBIG in patients who were at least 12 months post-liver transplant for HBsAg positive disease and who had been maintained on combination lamivudine and low-dose IM HBIG without evidence of hepatitis B recurrence. In Chapter five, the efficacy of combination adefovir and lamivudine as primary hepatitis B prophylaxis post-transplant was explored. Both studies recruited patients from the liver transplant centres in Victoria, New South Wale and New Zealand.

In the post-transplant study described in Chapter four, 34 patients were recruited and randomized to either switch from lamivudine/HBIG to lamivudine/adeovir (16 patients) or remain on lamivudine/HBIG (18 patients). Both groups were well matched for age, gender, time post-liver transplant (median of 4.5 years) and background virological risk for recurrence (~25% with detectable viral load at transplant and a median viral load prior to lamivudine approximately 5 log_{10} IU/mL). With a median follow-up of 22 months there were no cases of recurrence (defined as presence of HBsAg and detectable HBV in serum using a sensitive PCR-based assay) in either group. One patient was withdrawn from the adefovir/lamivudine group due to an increase in serum creatinine (this patient had pre-existing diabetic and hypertensive nephropathy) and was switched back to HBIG/lamivudine prophylaxis. Otherwise median creatinine did not change over the duration of the study and there were no other adverse events considered related to study therapy. This protocol was estimated to save 40% of the cost of post-transplant hepatitis B prophylaxis (>$AUD 5,000 per patient per year).

In the study of combination adefovir and lamivudine therapy described in Chapter five, 26 waitlisted patients were recruited of whom 12/26 patients had previous lamivudine exposure but none had lamivudine resistance. Median HBV viral load prior to institution of antiviral therapy was ~ 3.5 log_{10} IU/mL. 19/26 patients progressed to liver transplant and 800 IU IM HBIG was given at transplant and daily for seven subsequent days only (total HBIG dose 6400 IU). Of the remaining 7
patients who did not progress to transplant 2 patients were delisted for improvement in underlying liver function with antiviral therapy, 2 patients were delisted for progression of hepatocellular cancer beyond transplantable criteria, one patient died of cryptococcal sepsis and the final two patients remained on the transplant waiting list. All transplanted patients survive without recurrence (HBsAg negative and HBV DNA undetectable) with a median follow up of 23 months post-transplant. In the context of organ transplantation and calcineurin inhibitor immunosuppressive therapy median serum creatinine in these patients rose from 81 to 114 μmol/L over the course of the study and adefovir dosage was adjusted as necessary. However, no patient required adefovir cessation and there were no adverse events considered related to study therapy.

Taken in combination, these two studies support the replacement of HBIG as the cornerstone of post-transplant hepatitis B recurrence prophylaxis. This will significantly decrease the cost of post-transplant prophylaxis as well as improving the tolerability of prophylaxis for transplant recipients. In both these studies, further follow-up is required to confirm the long-term efficacy of post-transplant prophylaxis. Such follow-up is planned. In addition, one would expect that alternate antiviral agents (or combination of agents) with comparable or better antiviral properties would perform at least as well as lamivudine and adefovir. Indeed, given the availability of agents such as entecavir and tenofovir one can no longer justify the use of lamivudine or adefovir in treatment naïve patients and future studies should include these drugs given either alone or in combination.
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Title: Novel antiviral treatment strategies in chronic hepatitis B

Date: 2010

Citation: Patterson, S. J. (2010). Novel antiviral treatment strategies in chronic hepatitis B. Doctorate, Department of Medicine, Austin Health, The University of Melbourne.

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