Hepatitis C virus: the growing challenge

Brian J. Thomson*

Department of Microbiology and Infectious Diseases, School of Molecular Medical Sciences, University of Nottingham, Nottingham NG7 2UH, UK

Background: Hepatitis C virus (HCV) is a major cause of liver disease worldwide. In industrialized countries, intravenous drug users (IDUs) are the main reservoir of infection. Relatively little information is available on HCV in the developing world.

Sources of data: Peer reviewed publications and presentations at major academic meetings.

Areas of agreement: HCV-related cirrhosis and death from hepatocellular carcinoma are likely to rise dramatically in the next three decades. Urgent intervention is required both to minimize the burden of disease in those already infected and to reduce the incidence of new infections, particularly in the IDU population.

Areas of controversy: Current models of care and commissioning in the UK and other countries do not adequately identify or treat HCV infection in IDUs. Most strategies focus on disease prevention and do not target new infections.

Growing points: New models of care are currently being developed and validated.

Areas timely for developing research: The development of new models of HCV replication will transform our understanding and capacity to treat HCV infection.

Keywords: hepatitis C virus/treatment/models of care/pathogenesis

Introduction

Hepatitis C virus (HCV) is a major cause of end-stage liver disease and hepatocellular carcinoma (HCC) and is now the most common indication for liver transplantation.1 It is estimated that up to 170 million people worldwide are infected with the virus. The prevalence of HCV varies widely with geographical location and within populations. The overall prevalence of HCV is almost 2% in North America, 3–4% in some Mediterranean and Asian countries and more than 10% in parts of Central Africa and Egypt.2 In England and Wales, the most recent available estimates indicate that ~0.6% of the adult population aged 15–59 have antibodies to HCV.3 Although this represents a relatively
low prevalence in comparison to the USA and other European countries, the UK Health Protection Agency has estimated that almost 6000 infected individuals will develop cirrhosis, and up to 1500 will have end-stage liver disease or HCC, by 2010. These figures are likely to rise dramatically in the next three decades.\textsuperscript{4,5} HCV is therefore a major burden on healthcare resources in the UK and worldwide. The principal challenges to reducing this burden lie in the identification and delivery of care to the major infected populations, and the provision of more effective therapies for the eradication of HCV. This review will briefly describe the virology of HCV, and will then focus in more detail on these challenges.

Virology

HCV is a small, enveloped virus containing a single-stranded, positive sense RNA genome. It is extremely adept at evading both the innate and adaptive immune response and the majority of individuals exposed to HCV become chronically infected. It is classified in the Hepacivirus genus within the Flaviviridae family of viruses, which includes yellow fever virus and the animal pestiviruses.

The genome

The HCV RNA genome is approximately 9600 nucleotides in length. In common with other members of the flavivirus family, the viral genome is composed of a 5' non-coding region (NCR), a single, long open-reading frame with the potential to encode a polyprotein precursor of about 3000 amino acids and a 3' NCR. The HCV polyprotein is co- and post-translationally processed by cellular and viral proteases to yield the mature structural and non-structural proteins. HCV structural proteins include the core protein (p19 in its fully mature form) and the envelope glycoproteins E1 (p35) and E2 (p70). The structural proteins are separated from the non-structural proteins by the short membrane polypeptide p7. Non-structural proteins are designated NS2–NS5 and include: NS2 (p23), NS3 serine protease/RNA helicase (p70), the NS4A polypeptide (p8), NS4B (p27), NS5A phosphoprotein (p56–p58 depending on phosphorylation status) and NS5B (p68). The NS proteins and p7, which is reported to have ion channel activity, are essential for viral replication and have emerged as major targets for the design of novel antiviral agents.\textsuperscript{6} The function of each of these proteins is illustrated in Table 1.
Viral entry

HCV entry into the host cell is a complex and highly regulated process, which is not yet fully understood. Initial attachment of the virion to the cell may require the presence of both glycosaminoglycans and the low-density lipoprotein (LDL) receptor. Entry then proceeds through sequential interaction with the scavenger receptor class B type I, the tetraspanin CD81 and the tight junction proteins Claudin-1, -6 or -9.7 This multi-step process is consistent with the observation that most of these potential HCV receptors have been shown to be necessary, but not sufficient, for viral entry. It is likely, however, that the process of viral binding and entry varies with the details of the experimental model used and with the environment of the virus–host cell interaction in vivo. HCV circulates in infected patients as either ‘free forms’ or complexed with immunoglobulin or high-, low- or very low-density lipoproteins. There is evidence that the virus uses these serum-derived factors both to broaden its receptor specificity and to evade neutralizing antibody responses.8

Viral replication

HCV infection appears to be a highly dynamic process with a viral half-life of only a few hours and average daily virion production and clearance rates of more than $10^{12}$. These findings are similar to the
dynamics of HIV infection and provide a rationale for the use of multi-drug combination therapies against HCV.\textsuperscript{9} The kinetics of viral replication, together with the lack of a proof-reading function of the viral RNA-dependent RNA polymerase, provide the basis for the genetic variability of HCV. HCV isolates cluster into six major genotypes, which differ from each other by \(~30\%\) at the nucleotide and deduced amino acid level. Genotypes 1–3 have a worldwide distribution. Genotypes 4 and 5 are found principally in Africa and the Middle East, and genotype 6 is distributed primarily in Asia. Genotypes can each be sub-divided into a large number of subtypes. In addition to these categories, HCV exists within individuals as a constantly evolving population of closely related but distinct viruses known as a quasi-species. The presence of such a highly heterogeneous population facilitates selection of mutations by either the immune response or therapeutic interventions and is likely to be one of the main factors that enables HCV to persist for the lifetime of the host.

**Models of pathogenesis**

The pathogenesis of HCV infection remains incompletely understood. Until recently, there has been no robust small animal model for the study of HCV or \textit{in vitro} culture model for the propagation of infectious virus. \textit{In vitro} studies largely interrogated the effects of viral proteins in transformed cell lines. These studies, while of interest, often provided contradictory results of uncertain relevance to \textit{in vivo} infection. Recently, transgenic technology and the development of the uPA-SCID mouse chimera, in which mouse liver is reconstituted with human hepatocytes, have provided small animal models that can sustain HCV replication and show some features of human liver disease. Infection with the related GBV-B virus in new world monkeys has also been used as a basis for interrogating the pathogenesis of HCV. These models may accommodate pre-clinical toxicity and efficacy studies, but have not yet found widespread acceptance. The ‘gold standard’ model for investigating the pathogenesis of HCV and the potential of antiviral drugs continues to be the chimpanzee. There are compelling economic and moral reasons to limit the use of this primate in research. Further, chimpanzees do not develop severe liver disease in a manner analogous to humans and are therefore not ideal models. The development of alternative systems for the study of HCV therefore remains a major focus.

The first step in developing new models for the study of HCV replication and pathogenesis was the development of a system of sub-genomic segments of HCV (replicons) engineered to provide stable, high-level
expression of viral RNAs and structural and non-structural proteins. The sub-genomic replicon system, however, while a major advance, failed to couple RNA replication and virion assembly and did not produce infectious virus. A remarkable further development has been the generation of an infectious clone of a genotype 2a isolate of HCV known as JFH-1. JFH-1 has the unique capacity to undergo a full viral life cycle, with the production of infectious virus, in hepatocyte-derived cell lines. This clone has subsequently undergone adaptive mutation to permit the generation of increased titres of infectious particles in a hepatoma cell line. An intragenotype chimeric virus based on JFH-1 has enhanced infectivity in animal models. JFH-1 has subsequently been modified by intergenotypic recombination to produce infectious chimeric clones of viral genotypes 1–4.

In addition to this major advance, models have been developed for the interrogation of specific aspects of viral life cycle. For example, there has been, until recently, no good system to study the interactions between viral glycoproteins, cellular receptors and antibody in the process of viral entry. This has now been overcome by the development of pseudoparticles (HCVpp), consisting of unmodified HCV envelope proteins assembled on retrovirus core particles. HCVpp representative of genotypes 1–6 have been constructed. This methodology has been combined with infectious clones to identify antibodies with the capacity to inhibit infection by multiple HCV genotypes. Such broadly cross-neutralizing antibodies may be of direct clinical utility, and could be used to reduce the incidence of infection following needle stick injury or graft re-infection following liver transplantation. Experimental systems are also evolving to closely model the in vivo environment. Recently, for example, both the LDL receptor and CD81 have been shown to have a critical role in infection of primary human hepatocytes by serum-derived HCV. Taken together, these advances will transform our understanding of HCV pathogenesis and greatly facilitate HCV drug discovery.

Epidemiology

Intravenous drug abuse is the major risk factor for the acquisition of the virus and intravenous drug users (IDUs) constitute by far the largest pool of HCV infection in industrialized countries. Since the introduction of screening of the blood supply by both antibody and nucleic acid testing, new cases of post-transfusion HCV in the UK have virtually disappeared. Systematic screening of blood donors is, however, not universal, and new cases of post-transfusion HCV continue to occur in resource poor settings. The unintended transmission
of HCV during national campaigns, such as that for the parenteral treatment of schistosomiasis in Egypt, has also contributed to a national prevalence of up to 15% in some countries.19

In contrast to hepatitis B virus, sexual transmission of HCV is rare. A long-term prospective study of HCV among 895 monogamous heterosexual partners of individuals chronically infected with HCV, with a total follow-up period of more than 8000 person-years, found an extremely low or null risk of sexual transmission.20 In contrast, an epidemic of acute hepatitis C is emerging among HIV-infected men who have sex with men (MSM), with a growing number of cases reported in the MSM population in the USA and Europe. Transmission in this group appears to be permucosal rather than percutaneous, and correlates with the numbers of sexual partners, the sharing of drugs through the nasal or anal route and high-risk sexual practices.21 Most practitioners suggest that HCV-infected individuals use barrier methods of contraception, but do not recommend continuing such precautions in stable monogamous relationships.

Mother-to-infant transmission of HCV has been recorded with a risk in most studies of less than 5%. Women co-infected with HCV and HIV are more than twice as likely to transmit HCV to their children as those infected with HCV alone. The European Paediatric HCV Network recommends that, on the basis of current evidence, neither elective Caesarean section nor avoidance of breast feeding is of benefit in preventing HCV transmission from mother to child.22 Mothers co-infected with HCV and HIV should follow the existing HIV guidelines, which emphasize maternal choice but recommend elective Caesarean section at 38 weeks.

Natural history

The natural history and consequences of chronic infection with HCV have now been well described in several large-scale studies. HCV leads to cirrhosis and the consequent life-threatening development of end-stage liver disease or HCC in 20–30% of infected individuals over a lifetime. Co-factors for disease progression include increased age at infection, male sex and high alcohol intake.3 The evidence base for disease progression has informed robust models of the natural history of infection, which have been used to predict the future burden of disease. The number of HCV-infected individuals with compensated cirrhosis in the UK is estimated to rise from 3705 in 2005 to 7550 by 2015. The number with decompensated cirrhosis or HCC will also rise, to 2540 by 2015, with further substantial increase predicted.4 A similar experimental approach in the USA predicted that deaths from
HCV will rise from 3700 in 1998 to 13 000 by 2030. It is striking that the same model predicted that deaths from HIV in 2030 will reach 4200. Consistent with this discouraging outlook, cohort studies suggest that individuals with severe liver fibrosis secondary to hepatitis C may have a worse prognosis than those with fibrosis of other aetiologies.

HCC is a particularly important complication of HCV infection. The 5-year cumulative incidence of HCC in HCV-infected individuals is 17% in Western countries and 30% in Japan, higher than in cirrhosis of any other aetiology. As a comparison, the 5-year cumulative incidence of HCC is ~8% in alcoholic cirrhosis and 4% in cirrhosis of auto-immune aetiology. The high prevalence of HCV is likely to have contributed to the increased frequency of HCC in the USA.

Treatment

Established treatment guidelines for chronic HCV infection comprise either a 24- or 48-week course of pegylated interferon and ribavirin, depending on viral genotype. Using these regimens, a sustained viral response (SVR), as defined by the absence of HCV in the peripheral blood 24 weeks after the end of treatment, has been reported in randomized, controlled trials (RCTs) in 42–52% of patients with HCV genotype 1 disease and in 76–82% of those infected with genotype 2/3 virus. Although RCTs are important in establishing efficacy, the development of strategies for the treatment of HCV also requires an assessment of the effectiveness of the intervention in routine clinical practice. A recent study addressed the efficacy of antiviral therapy in 347 patients treated with pegylated interferon and ribavirin, according to current guidelines, in secondary care clinics in the Trent region of the UK; 37.2% of those with genotype 1 infection and 70.1% with genotype non-1 infection achieved SVR. In addition to viral genotype, factors predictive of a response to therapy were age at starting treatment and disease stage on pre-treatment liver biopsy. This study also used multivariate logistic analysis to predict the probability of SVR for sub-groups defined by disease stage, genotype, and age at commencement of therapy. This model revealed striking differences in predicted response rates between sub-groups and provided a strong rationale for early treatment, particularly of those with genotype 1 disease (Table 2). These results differ from those of another large ‘real world’ study from the UK, in which Dudley et al. reported only a 28% response rate in genotype 1 patients treated in a clinical setting. However, a higher proportion of patients had cirrhosis on pre-treatment liver biopsy in the study by Dudley et al. than in the Trent
Further, 23% of those reported by Dudley failed to complete therapy, in comparison to only 11% in the Trent study. Taken together, these factors are likely to account for the difference in outcome between the two studies and emphasize the importance of the patient population in determining the outcomes of treatment. Recently, it has been established that patients with genotype 1 or genotype 2/3 disease, who have no detectable virus in the peripheral blood after 4 weeks of combination therapy (‘super responders’), can reduce their treatment duration by 50% without loss of efficacy.28,29

**HCV—does it matter?**

Although the natural history of HCV infection has recently been clarified and used to inform mathematical modelling3–5, the consequences of chronic infection and the long-term benefits of antiviral treatment are surprisingly poorly quantified. These issues have previously been studied in selected population sub-groups, such as those who acquired HCV through blood transfusion, or in young women infected by anti-D immunoglobulin. Data on the morbidity and mortality associated with HCV infection are important in the rational development of national strategy and it is essential to establish the morbidity and mortality associated with chronic HCV infection in cohorts representative of the infected population. A recent study analysed a cohort of 2285 patients attending secondary care clinics in the Trent Region of England.30

**Table 2** Predicted probability of a sustained viral response to combination therapy for HCV infection analysed by sub-group.

<table>
<thead>
<tr>
<th>Biopsy score at start of treatment</th>
<th>Genotype</th>
<th>Age at treatment (years)</th>
<th>30</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Probability</td>
<td>95% CI</td>
<td>Probability</td>
</tr>
<tr>
<td>Mild</td>
<td>1</td>
<td></td>
<td>0.72 (0.54, 0.85)</td>
<td>0.57 (0.42, 0.70)</td>
<td>0.40 (0.24, 0.57)</td>
</tr>
<tr>
<td>Non-1</td>
<td></td>
<td></td>
<td>0.84 (0.75, 0.91)</td>
<td>0.82 (0.74, 0.89)</td>
<td>0.80 (0.70, 0.88)</td>
</tr>
<tr>
<td>Moderate</td>
<td>1</td>
<td></td>
<td>0.54 (0.29, 0.77)</td>
<td>0.37 (0.21, 0.55)</td>
<td>0.22 (0.12, 0.38)</td>
</tr>
<tr>
<td>Non-1</td>
<td></td>
<td></td>
<td>0.73 (0.57, 0.84)</td>
<td>0.70 (0.58, 0.80)</td>
<td>0.67 (0.55, 0.77)</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>1</td>
<td></td>
<td>0.20 (0.03, 0.69)</td>
<td>0.11 (0.02, 0.50)</td>
<td>0.06 (0.01, 0.34)</td>
</tr>
<tr>
<td>Non-1</td>
<td></td>
<td></td>
<td>0.43 (0.24, 0.66)</td>
<td>0.40 (0.24, 0.59)</td>
<td>0.37 (0.22, 0.55)</td>
</tr>
</tbody>
</table>

The table shows the predicted probability of achieving a sustained viral response (SVR) as assessed by multivariate logistic regression for sub-groups (disease stage, viral genotype and age at commencement of therapy). Gender is set to the average characteristics of the sample (66% male). Confidence intervals are reported on the probability scale (0–1). Thus, a 30-year-old male with mild liver disease and genotype 1 infection has more than 70% chance of achieving SVR, whereas a 50-year-old male with cirrhosis and genotype 1 disease has less than 10% of SVR. Data taken from Thomson et al.26. Reproduced with kind permission.
This cohort matched the demographics of HCV-infected populations elsewhere and was considered representative of the HCV-positive population in the UK. The principal finding of this study was that HCV-infected persons have a death rate three times higher than that of the age-matched general population. Excess mortality was due to both liver-related causes and the consequences of chronic drug use (Fig. 1). Predictors of liver-related mortality were age, treatment status, the degree of scarring on liver biopsy and mean alcohol consumption. A community-based study from Australia assessed mortality in HCV patients using a complementary but different methodology, also showed a death rate three times higher in those with HCV infection than in the general population. Excess mortality was a product of both drug-related and the liver deaths. Taken together, these two recent studies provide powerful evidence that HCV infection is an important and independent cause of death, even in those with high rates of co-morbidity.

In contrast to the clear evidence on mortality, the effect of treatment on survival rates was difficult to analyse conclusively. Treatment regimens have changed over time, as have the criteria by which patients qualify for treatment. Overall, however, completion of a course of therapy was associated with increased survival from all-cause and liver-related mortality. Current regimens would be expected to be of greater benefit than previous, less effective treatments.

![Fig. 1](https://academic.oup.com/bmb/article-abstract/89/1/153/360979)

The histogram shows the category of death by age for HCV-infected patients in the study by Neal et al. Numbers of deaths within each category are indicated. Age category is shown on the y-axis. Reproduced by kind permission.
Areas of controversy

Acute infection

In contrast to the substantial body of evidence describing the natural history and consequences of chronic HCV infection, there are major gaps in our understanding of acute disease. Acute infection can be defined as new HCV viraemia in the absence of detectable antibodies. Most patients acutely infected with HCV become antibody positive within 3 months, although seroconversion can sometimes take 6 months or longer. Acutely infected patients are often asymptomatic and may not present for medical care. Further, the majority of incident infections occur in IDUs, who as a group do not readily access medical services. There are therefore very few good prospective studies of the epidemiology and natural history of acute HCV infection. As a consequence, there are limited data on the incidence of acute infection and the precise behaviours that correlate with the new acquisition of HCV. A recent systematic review of acute infection identified 675 individuals who presented with acute hepatitis or acquired HCV following transfusion of infected blood or needle stick injury. Clearances rates, as defined by loss of HCV RNA within 6 months of exposure, varied in individual studies from 0% to 80%, with a mean of 26%. Clearance was not related to either age at infection or viral genotype, but was less common among males than females. Higher clearance rates were reported among those who presented with acute hepatitis than those who did not (31% versus 18%). Other studies have found that the strength and nature of HCV-specific CD4-positive T-cell responses mediate viral clearance and this observation may explain the correlation between clearance and acute hepatitis, which is likely to be at least in part the result of cellular immune responses to HCV in the liver.

A better understanding of the epidemiology and natural history of acute HCV infection is an important challenge for several reasons. First, changes in the incidence of acute HCV infection is an early measure of the impact of policy changes on transmission rates. Identification of new infections also introduces the possibility of patient education and contact tracing, with the potential to limit the spread of the disease at source. This is particularly important as acute HCV responds much better to treatment than more established disease. There are as yet no national guidelines for the treatment of acute HCV, but sustained viral responses of up to 95% have been achieved using 12 weeks of therapy with interferon alone. Finally, mathematical modelling has emphasized the importance of achieving a reduction of up to 80% in incident infection if the
HCV epidemic is to be controlled. It is difficult to envisage how such a reduction could be achieved by the current practice in the UK, which is focused on the prevention of disease progression by identification and treatment of individuals already chronically infected with HCV.

**Treatment of IDUs**

Intravenous drug use is the major risk factor for the acquisition of HCV and IDUs constitute by far the largest pool of HCV infection in the UK and other industrialized countries. Prevalence varies between 27% and 74% in different cohorts. Despite these very high rates of infection, antiviral therapy has not been consistently offered to this population. IDUs often do not attend hospital clinics regularly, and are perceived to be at greater risk from illicit drug use than HCV infection. Active IDUs are also at theoretical risk of re-infection and may not therefore represent a cost-effective use of high cost combination therapy. Until recently, these concerns informed national guidelines for treatment, including those in the UK. The most recent Practice Guidelines from the American Association for the Study of Liver Disease and current UK NICE guidelines, however, suggest that treatment for HCV should be offered to current injectors. It is, however, clear that the current hospital-based services do not effectively identify or treat HCV in IDUs. A UK study found that, of 61 new diagnoses identified by Drug and Alcohol services, only a single patient ever received antiviral therapy. New strategies are therefore required to engage and treat this extremely important population. Previous work has demonstrated the feasibility of treating HCV in IDUs who attend a secondary care clinic, but did not address the barriers to managing infection in this population. Two recent studies have, however, reported a community-based approach to the treatment of HCV in substance misusers in Australia and Canada. These studies suggest that treatment for HCV can be safely and effectively delivered to IDUs in a community setting by a multidisciplinary team including drug workers, psychiatrists and specialists in the treatment of HCV.

Overall, there is no doubt that the principal reservoir of HCV epidemic in the UK and other industrialized countries lies in populations of IDUs. Strategies for the reduction of the burden of HCV infection in IDUs are therefore essential. It is likely that such strategies will require a shift in national policy from secondary-based models of care to community-based delivery of harm reduction, prevention and treatment for the HCV epidemic.
Areas for developing research

New treatments for HCV

The present combination regimens for the treatment of HCV result in SVR in 76–82% of those infected with HCV genotype 2/3, but only 42–52% response rate in patients with HCV genotype 1 disease. There has therefore been a strong drive to find new and more effective treatments for HCV. These efforts have focused primarily on small molecule inhibitors of the NS genes of HCV (Table 1). The capacity to identify and characterize new agents has been transformed by the advent of the in vitro replicon systems.40 The replicon system has permitted the detailed study of viral and host genes necessary for the production of infectious virus and the very rapid assessment of the efficacy of new antiviral agents. The replicon systems also facilitates early analysis of viral resistance, cross-sensitivity studies and assessment of the efficacy of combination therapy and has been adapted for high-throughput screening of new drugs.41 The current systems are, however, based on a limited number of reference strains and do not accommodate the wide genetic variability of clinical isolates. This limitation has recently been addressed by the adaption of a genotype 1b-based replicon to permit the study of NS3 proteins derived from clinical isolates.42 Further, in vitro systems do not permit assessment of systemic toxicity and despite early optimism, several drug discovery programmes have failed. GlaxoSmithKline recently announced cessation of further studies of their acyl pyrrolidine NS5B RNA polymerase inhibitors due to pre-clinical toxicity data. This follows several high-profile failures of other drugs that had reached phase 2 clinical trials, but were withdrawn because of systemic toxicity, including the NS3 protease inhibitor BILN 2061 (Boehringer Ingelheim), the NS5B RNA polymerase inhibitors Valopicitabine (NM 283, Idenix) and HCV 796 (ViroPharma and Wyeth) and the NS4a inhibitor GS9132 (ACH-806, Gilead Sciences/Achillion Pharmaceuticals). The most advanced of the small molecule inhibitors is the NS3/4A protease inhibitor VX950 (Telaprevir, Vertex); 28 days of Telaprevir has recently been shown to improve rapid viral response rates when used in combination with pegylated interferon and ribavirin, with an acceptable safety profile.43 It is notable that the use of small molecule inhibitors as monotherapy has also been associated with the rapid emergence of viral resistance. It is therefore likely that drug regimens will, for sometime, continue to be based on interferon and ribavirin, but that at least some of the agents currently in development will lead to improved outcomes when used in combination, and may provide effective alternatives in the foreseeable future.
Conclusions

The HCV epidemic is still growing in importance. Although the incidence of HCV is falling in some countries, the burden of disease arising from the pool of chronic infection continues to rise. It has been estimated that, by 2030, HCV will cause substantially higher morbidity and mortality in industrialized countries than HIV. Urgent intervention is required to prevent this outcome. Major barriers, however, remain to the delivery of effective care for HCV, particularly to populations of IDUs where the prevalence and incidence of HCV is highest. Investment in more effective treatments for HCV has so far met with limited success. There are, however, grounds for optimism. National strategies are beginning to take account to the need to access and treat HCV in IDUs and new models for the more effective delivery of care are currently being validated. Further, novel model systems for the development and screening of HCV drugs will lead to the provision of new tools with which to reduce the burden of this important disease.

References


36 NICE Technology Assessment number 106; TA106. www.nice.org.uk/TA106guidance