Resistance to hepatitis C virus: potential genetic and immunological determinants

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Studies of individuals who were highly exposed but seronegative (HESN) for HIV infection led to the discovery that homozygosity for the Δ32 deletion mutation in the CCR5 gene prevents viral entry into target cells, and is associated with resistance to infection. Additionally, evidence for protective immunity has been noted in some HESN groups, such as sex workers in The Gambia. Population studies of individuals at high risk for hepatitis C virus infection suggest that an HESN phenotype exists. The body of evidence, which suggests that protective immunity allows clearance of hepatitis C virus without seroconversion is growing. Furthermore, proof-of-principle evidence from in vitro studies shows that genetic polymorphisms can confer resistance to establishment of infection. This Review discusses the possibility that genetic mutations confer resistance against hepatitis C virus, and also explores evidence for protective immunity, including via genetically programmed variations in host responses. The data generally strengthens the notion that investigations of naturally arising polymorphisms within the hepatitis C virus interactome, and genetic association studies of well characterised HESN individuals, could identify potential targets for vaccine design and inform novel therapies.

Introduction

Infectious diseases are a leading cause of human morbidity and mortality, and therefore act as a major selective pressure on the evolution of the human genome. Accordingly, clear evidence suggests that genetic variation in human populations contributes to a population’s susceptibility to infectious diseases, and in some individuals confers resistance to establishment of infection. This resistance is best exemplified by the recognition that individuals who are homozygous for a common loss of function variant of the FUT2 gene do not express the H type-1 oligosaccharide ligand needed for Norwalk virus binding, and cannot be infected with this pathogen.

Once infection is established, interactions between determinants of microbial virulence and host immune defence mechanisms underpin the pathogenesis of the infectious disease. The desired outcome for the host is efficient elimination of the pathogen, with restricted tissue injury, and long lasting immunological protection against reinfection. Conversely, the ideal outcome for the evolutionary survival of the pathogen is to ensure transmission to another host—ie, by induction of restricted tissue injury and infection of sufficient duration to ensure transmission to a new host. The generation of protective immunity against pathogens is clearly, at least in part, genetically defined.

Hepatitis C virus infection is a major problem for public health programmes worldwide, with an estimated 180 million individuals infected. Acute hepatitis C virus infection is usually asymptomatic and results in clearance in 25% of individuals infected. For chronically infected individuals, the virus drives sustained hepatic necro-inflammation and fibrosis, and persists throughout life unless cured by antiviral treatment. Transmission of hepatitis C virus is predominantly associated with sharing and re-use of injecting apparatus among injecting drug users (IDUs), but other parenteral means can cause transmission too, including tattooing and blood transfusion (before the introduction of serological screening globally in the early 1990s).

Hepatitis C virus encodes very few proteins and therefore depends heavily upon host factors for propagation (figure 1). Viruses enter hepatocytes via interactions between viral envelope proteins E1 and E2, and four known host receptors, CD81 (a widely expressed tetraspanin), scavenger receptor class B type-1, and the tight junction proteins claudin-1 and occludin. Additionally, other molecules including heparan sulfate, both dendritic cell and liver specific intracellular adhesion molecule-3-grabbing non-integrins, low-density lipoprotein-receptor, and the Niemann–Pick C1-like 1 cholesterol absorption receptor have all been implicated in hepatitis C virus cell attachment and entry. Investigators using infectious cell culture systems with both hepatitis C virus replicons (which do not contain the full genome) and the full-length-genome Japanese fulminant hepatitis-1 strain, have identified hundreds of host-viral protein interactions, many potentially relevant to resistance and protective immunity (figure 2). For example, RNA interference (RNAi) screens have uncovered many host cofactors for hepatitis C virus replication, with phosphatidylinositol 4-kinase-IIIa (PI4K-IIIa) the most consistently identified factor. This enzyme phosphorylates phosphatidylinositol in the 4 position of the inositol ring to generate phosphatidylinositol 4-phosphate. PI4K-IIIa kinase activity is needed for membranous web formation, and silencing of PI4K-IIIa results in an aggregation of double-membrane vesicles and hepatitis C virus replication complexes. AL-9, a member of the 4-anilinoquinazoline-containing kinase inhibitor family, inhibits hepatitis C virus replication in vitro by directly inhibiting PI4K-IIIa. Another RNAi screen targeting about 4000 human genes identified nine cellular genes that regulate viral replication. Investigators silencing these genes reported an inhibition of viral replication by more than 60%.
The expression of many host cellular proteins, which might modulate hepatitis C virus replication, such as heat shock protein \(22^{27}\), \(\alpha\)-actinin,\(^{23}\) nucleolin,\(^ {24}\) eukaryotic initiation factor 4A-I,\(^ {25}\) and Rho GDP-dissociation inhibitor 2\(^ {26}\) can be increased in the presence of replicating hepatitis C virus. Prominent in this list too are host immune response proteins, notably including the interferon-stimulated genes such as viperin, which interacts with both hepatitis C virus NS5A, core proteins, and vesicle-associated membrane protein-associated protein subtype A, which results in the disruption of viral replication.\(^ {27}\) Additionally, strong evidence exists for a role of apolipoprotein E as a hepatitis C virus infectivity factor because viral replication is closely associated with cellular lipids.\(^ {28,29}\) Small interfering RNAs or inhibitors that target components of very low density lipoprotein synthesis, inhibit infectious hepatitis C virus secretion.\(^ {28,30,31}\)

Much remains to be discovered in relation to the hepatitis C virus-host protein interactome and the host immune response characteristics against this pathogen. Nevertheless, evidence suggests that hepatitis C virus replication is dependent on many host factors and investigation of genetically defined variations in these host proteins could uncover mechanisms of resistance and protection against hepatitis C virus infection.

**Evidence for resistance and protection against hepatitis C virus infection**

**Identification of individuals who are highly exposed but seronegative**

Injecting drug use is the major risk factor for hepatitis C virus transmission in developed countries, with seroprevalence in cross-sectional studies of IDUs ranging from 50% to more than 90%.\(^ {32}\) A few long-term IDUs remain seronegative and aviraemic, despite extended and probably repeated exposure to hepatitis C virus through sharing of drug-injection equipment.\(^ {33}\) This highly exposed but seronegative (HESN) group have been termed exposed uninfected, which suggests the absence of demonstrable infection, as defined by conventional antibody or RNA testing.\(^ {34}\) Several prospective studies of high-risk IDUs have likewise reported subgroups that have remained seronegative despite longstanding high-risk behaviour,\(^ {35}\) who might represent a phenotype that are resistant, or became infected but had efficient viral clearance before seroconversion.\(^ {36}\) A quantitative meta-analysis of the prevalence of hepatitis C virus infection in relation to time since onset of injecting-drug use, showed both linear and quadratic effects (figure 3), with a 95% CI of hepatitis C virus prevalence ranging between 93% and 99% after 15 years of use.\(^ {37}\) In view of the apparent
plateau in the prevalence datasets, some or all of the remaining 1–7% of the group are plausibly HESN individuals.

In February, 2014, we reported a framework for reliable identification of the HESN phenotype in a prospective cohort of Australian IDUs in prison to allow investigation of genetic determinants of resistance and protective immunity. A risk–behaviour algorithm predicting incident infection was developed, similar to the procedure used to model coronary heart disease risk and HIV

Figure 2: Network diagram showing connections between HCV proteins, HCV-interacting human proteins, and candidate proteins from the HCVcc siRNA screen

The appendix shows a summary of the HCV interactome. Reproduced from Li and colleagues, by permission from National Academy of Sciences.

HCV (cc)=hepatitis C virus (cell culture system).

See Online for appendix
infection risks. The index includes many recognised behavioural risk factors for transmission (eg, injecting drug use, sharing of injecting apparatus, and injecting heroin) weighted on the basis of the individual hazard ratio for prediction of incident infection. Application of this index at each timepoint to longitudinal data about risk behaviours in the cohort showed that a small subset of individuals in the upper risk tertile had consistently raised risk indices, comparable with, or higher than, the incident cases, but remained uninfected (figure 4). Although this algorithm and the associations identified need to be replicated in other cohorts, this approach offers a framework for improved reliability in the definition of HESN for future studies.

Protective immunity against hepatitis C virus

Primary infection

If researchers are able to understand the differences in immune response characteristics between individuals who clear chronic infection and those who develop it, they will gain insights into potentially protective immunity because acute hepatitis C virus infection results in clearance in 25% of cases, and is associated with innate and adaptive immune responses in most individuals. However, reinfection in high-risk individuals is common, including rapidly after an initial episode, suggesting that the immunity induced by primary infection generally has restricted cross-protective capacity.

Soon after hepatitis C virus infection, an innate immune response is evident in the liver and in the blood, featuring induction of antiviral proteins (notably type 1 interferons α and β) and thereafter many interferon-stimulated genes with antiviral properties. Increased interferon-stimulated gene expression in the liver during acute hepatitis C virus has been associated with spontaneous clearance. Natural killer (NK) cell activation has likewise been shown to predict clearance, although activation has been reported irrespective of outcome.

Early, vigorous, and sustained CD4-positive T-cell proliferative responses against many hepatitis C virus proteins predict disease resolution. In-vivo depletion studies in chimpanzees have shown that virus-specific CD8-positive T cells and CD4-positive T cells are key effector cells for the host when controlling hepatitis C virus replication. During primary infection, CD8-positive T cells often show an exhausted phenotype with impaired ability to secrete interferon γ. Additionally, a close reciprocal association exists between CD8-positive T-cell exhaustion and viral escape. Neutralising antibodies that block hepatitis C virus cell entry are directed against epitopes in the E2 region of the envelope, including the hypervariable regions. Studies that used hepatitis C virus pseudoparticles to model systems enabled the investigation of virus entry and showed that neutralising antibodies are sometimes induced in the early phase of infection in patients who subsequently clear the virus. Conversely, in the chronic progressors (analogous to HIV infection), these antibodies typically appear late and have neutralisation activity against variants no longer present in the quasispecies.

Reinfection

Early studies in the chimpanzee model showed that repeated exposure to homologous and heterologous strains of hepatitis C virus could result in repeated infection, although reinfection was generally associated with reduced periods of viraemia and a heightened probability of clearance. Reinfected chimpanzees had rapid acquisition of specific cytolytic activity by liver resident CD8-positive T cells and expansion of memory CD4-positive and CD8-positive T cells in the blood, featured reduced peak amounts of alanine aminotransferase, and produced interferon γ and tumour necrosis factor α (TNFa) earlier and at higher amounts than normal. This phenotype has been confirmed in human beings. For instance, individuals with primary infection were reported to be 12-times more likely to develop chronic infection, and have average hepatitis C viral loads 2 logs higher than did those who became infected after having previously cleared hepatitis C virus. However, a study in 2013 of hepatitis C virus preexposed chimpanzees did not reproduce this finding. The chimpanzees were repeatedly exposed to human plasma with trace amounts of hepatitis C virus, and had induction of hepatitis C virus-specific T cells without seroconversion and systemic viraemia, but were not protected upon subsequent hepatitis C virus challenge. Conversely, the investigators reported suppression of the
chimpanzees’ immune responses, and two of three pre-exposed chimpanzees developed chronic infection after rechallenge with the virus, with concomitant regulatory T-cell expansion. An important point to emphasise is that the inoculum of hepatitis C virus infection is often not known in studies on human cohorts. Another study reported that only five of nine patients who had successfully eliminated a previous hepatitis C virus infection were able to spontaneously resolve a subsequent infection. Spontaneous resolution of reinfection was associated with a rise in both the magnitude and breadth of the total hepatitis C virus-specific T-cell response, suggesting generation of de-novo T-cell responses.

HESN phenotype

Cross-sectional studies have documented hepatitis C virus-specific CD4-positive and CD8-positive T-cell responses in the absence of viral antibodies or viremia, in a range of high risk groups who remain uninfected. These studies include those of family members of patients with chronic hepatitis C virus, sexual partners of people with chronic hepatitis C virus, health-care workers with needlestick injuries, children born to chronically infected mothers, children living with hepatitis C virus-infected siblings, and high-risk uninfected IDUs. NK cell activity might also have a role in abrogating of established infection in high-risk uninfected IDUs, and in infants born to chronically infected mothers. Additionally, one report has shown hepatitis C virus-specific cytotoxic T-cell responses in conjunction with established, but ultimately transient, viremia without seroconversion, in prisoners who acknowledged use of injecting drugs and sharing of the injecting apparatus. Similar findings were reported in chimpanzees inoculated with very low doses of hepatitis C virus.

Demonstration of CD8-positive cytotoxic T-cell responses against viral antigens is usually assumed to show established infection, leading to processing and presentation of viral peptides through class I MHC. Hence, detection of hepatitis C virus-specific cytotoxic T-cell responses in HESN individuals potentially suggests that these individuals have been previously infected with hepatitis C virus. Previous infection might also be associated with rapid seroreversion. Follow-up studies after spontaneous clearance suggest that 20–50% of individuals serorevert, to become hepatitis C virus antibody negative more than a decade after spontaneous viral clearance. Alternatively, T-cell responses to not previously encountered antigens can occur, and have been associated with enhanced or diminished immunity and changed immunopathological effects. In hepatitis C virus, a cross-reactive epitope is shared between the NS3 protein and the influenza virus, and CD8-positive T-cell responses, specific for this epitope, have been reported in acute hepatitis C virus and associated with severe illness.

Occult infection

Another plausible explanation for the HESN phenotype is that these individuals are infected with a replication-defective viral variant, or one with a non-hepatocyte cellular tropism, allowing generation of cellular immunity without seroconversion or evidence of viremia in the circulation. Such so-called occult hepatitis C virus infection has been reported in B and T lymphocytes, dendritic cells, and monocytes from patients who have cleared the virus, albeit at very low proportions. This occult hepatitis C virus infection might be analogous to so-called elite controllers of HIV infection, in which some individuals harbour HIV proviral DNA in resting CD4-positive T cells at proportions 10⁴–10⁶ times lower than those in most infected individuals, which is much lower than the detection levels of conventional assays. This low degree of infection might plausibly be beneficial to the host by providing persisting antigenic stimulation to sustain or enhance both innate and either HIV or
hepatitis C virus specific cellular immunity, thereby reducing the possibility of reinfection.\textsuperscript{80,81}

Genetically determined resistance against hepatitis C virus infection

Substantial genetically determined resistance to HIV infection with the macrophage-tropic HIV variants, which predominate in transmission, was proven to be conferred via homozygosity for the Δ32 truncation mutation in the CCR5 gene, which acts as a major co-receptor for HIV entry.\textsuperscript{84} This discovery underpins the notion of a similar scenario in relation to hepatitis C virus. However, resistance to hepatitis C virus infection in HESN individuals is largely unexplored. The proof-of-principle for such genetic resistance is evidenced by in-vitro studies examining host proteins, which interact with hepatitis C virus. Cyclophilin A, a member of a family of cellular peptidyl-prolyl-isomerases, is a host-encoded factor that is essential for hepatitis C virus replication and possibly particle assembly too.\textsuperscript{85} Peptidyl-prolyl-isomerase catalyses the isomerisation of peptide bonds from the trans to the cis form at proline residues and helps with protein folding. Several hepatitis C virus non-structural proteins (NS2, NS5A, and NS5B) have been reported to interact with cyclophilin A,\textsuperscript{86} and cyclophilin A mutants without isomerase activity do not sustain viral replication.\textsuperscript{85} A report\textsuperscript{87} in 2012, showed that homozygosity at any of the three naturally arising single nucleotide polymorphisms in the region of the peptidyl-prolyl-isomerase gene, resulted in an unstable cyclophilin A protein, intracellular cyclophilin A depletion, and a hepatitis C virus refractory phenotype in vitro. However, our 2014 study\textsuperscript{88} of the potential association between these single nucleotide polymorphisms and the HESN phenotype in 210 Australian prisoners who injected drugs reported that no participants were homozygous for the minor allele.

Claudin-1 is a coreceptor needed for late stage binding of hepatitis C virus to hepatocytes.\textsuperscript{89} A study\textsuperscript{90} of 68 IDUs who were not infected with hepatitis C virus reported that these individuals carried single nucleotide polymorphisms –15312C, –7153A, and –5414C in the claudin-1 promoter region more often than individuals with chronic hepatitis C virus infection (n=658) and hepatitis C virus clearers (n=199).

Resistance in HESN individuals might also be attributable to genetic variations in immunological proteins, which might contribute to very efficient clearance of established infection, thus representing genetically determined protection. In HIV, HESN individuals are more likely to carry specific MHC class I and II alleles than are seropositive individuals,\textsuperscript{91} notably a cluster of closely related HLA alleles (the A2 or 6802 supertype).\textsuperscript{92} These alleles are known to present the same peptide epitopes for T-cell recognition. Additionally, heterozygosity for the NK cell expressed killer inhibitory receptor (KIR) genotype (2DL2 or 2DL3), in combination with the absence of the HLA-C1 ligand for this KIR, has been reported to be associated with HESN in African sex workers.\textsuperscript{93}

Furthermore, in HIV the characteristics of potentially protective immunity, particularly in HESN sex workers in Nairobi, Kenya, have been a focus of investigation.\textsuperscript{94} Rowland-Jones and colleagues\textsuperscript{95} postulated that these women were initially exposed to viral inocula that efficiently primed a cell-mediated immune response in the absence of antibody synthesis. Consistent with this notion, HESN individuals have been reported to have polarised Th1 cytokine responses;\textsuperscript{96} cytotoxic T-cell responses to both HIV envelope and gag epitopes;\textsuperscript{97} enhanced NK cell production of interferon γ, TNFα, and the chemokine ligands for the CCR5 receptor, CCL3, CCL4, and CCL5,\textsuperscript{98} and diminished cell surface expression of the major chemokine coreceptors for viral entry CCR5 and CXCR4.\textsuperscript{99} Notably, several of these women subsequently became infected after a period of reduction in the frequency of, or an interruption to, sex work, suggesting that the initial protective mechanisms were maintained by repeated exposure to HIV.\textsuperscript{100}

In hepatitis C, a well characterised combination of genetic and environmental factors account for much of the varied probability of clearance from primary infection. The key genetic factors are single nucleotide polymorphisms in the IFNL3 gene locus, which have been associated with both spontaneous clearance\textsuperscript{101–105} and clearance via interferon-based antiviral treatment.\textsuperscript{106} The IFNL3 locus encodes interferon λ 3, which shares a common signalling pathway with type I interferons.\textsuperscript{107} Genome-wide association studies\textsuperscript{108} have shown that these three times differences in the rates of spontaneous hepatitis C virus clearance in diverse ethnic groups, such as African–Americans and Japanese individuals, are probably caused by the frequency of these IFNL3 single nucleotide polymorphisms. In 2013 a new transcript encoding an interferon γ 4 protein was identified upstream of the IFNL3 locus, and clearance was strongly associated with the interferon γ 4-TT variant, which is in strong linkage disequilibrium with the other clearance-associated single nucleotide polymorphisms.\textsuperscript{109–112}

A report\textsuperscript{113} in 2011 identified HESN individuals as having a significantly lower frequency of the protective IFNL3 genotype than anti-hepatitis C virus-positive spontaneous resolvers, but a similar frequency to patients who were chronically infected. Another report\textsuperscript{114} in HESN individuals suggested the same association, but did not include infected participants as a comparator. Our study\textsuperscript{115} in well characterised HESN individuals, uninfected low risk individuals and incident cases in the Australian IDU prisoner cohort reported no association.

The association between HLA alleles with spontaneous hepatitis C virus clearance has been studied extensively, in view of the evidence for CD4-positive and CD8-positive T-cell responses in clearance of primary infection.\textsuperscript{116}
(see appendix for a summary of the HLA associations of spontaneous hepatitis C virus clearance). The class I alleles HLA-A*1101, HLA-A*03, HLA-B*57, HLA-B*27, and HLA-Cw*0102 have all been associated with clearance. HLA-B*57 and HLA-B*27 are of particular interest in view of their protective effect in HIV, with over representation of HLA-B*57 in HIV-elite controllers, and a consistent association of HLA-B*27 with a more favourable course of untreated HIV infection.109 However, the most widely reported associations with spontaneous hepatitis C virus clearance are with HLA class II alleles, such as HLA-DRB1*03 and HLA-DQB1*0301.110 No association between HESN and either class I or II alleles was reported in the Australian IDU prisoner cohort, although the small sample size of 210 individuals, including eight HESN individuals, did not exclude a possible association.18

Associations between KIR loci and the outcome of acute hepatitis C virus infection have also been reported. The KIR locus shows substantial population diversity,111 with different individuals harbouring varying numbers of KIR genes, and wide-ranging allelic polymorphisms.112 KIR proteins react with one of two epitopes at aminoacid position 80 on HLA-Cw (and positions on a few rare HLA-B) molecules. An association between individuals homozygous for KIR2DL3 (an inhibitory receptor gene) and HLA-C1 (KIR2DL3’s ligand gene) and spontaneous clearance of acute hepatitis C virus infection has been reported.113 This receptor–ligand combination could provide weaker inhibitory signals than could other inhibitory KIR–HLA-C receptor–ligand pairings, and thus prime a more responsive NK cell phenotype.114,115

Knapp and colleagues116 studied 29 HESN IDUs from needle exchange or community drug services and 19 from a correctional centre, and compared these individuals with 257 patients with chronic hepatitis C virus infection. The HESN IDUs group included individuals who had a broad range of injecting duration (0–3–24 years) and lifetime injection episodes (36–17 520). Of the putative HESN individuals, only 27% reported present intravenous drug use and 100% reported sharing of needles or other drug injection equipment. No other selection criteria were applied to the group. A greater frequency of homozygosity for the KIR2DL3/HLA-C alleles was noted among the HESN group (25–0% vs 9–7%, odds ratio 3–1).117 By contrast, no association was reported in the Australian IDU prisoner cohort; again, the small sample size did not exclude a possible association.18

Many associations between polymorphisms in cytokine genes and spontaneous hepatitis C virus clearance have been reported; however, none has been consistently reproduced. Interleukin 12 is a heterodimer of p35 and p40 subunits and is a key cytokine in promotion of antiviral Th1 responses. The gene encoding interleukin 12 p40 (IL12B) is polymorphic and located on 5q31–33, and a functional single nucleotide polymorphism (A→C) of the 3’ untranslated region at position 1188 has been identified.118 The homozygous CC genotype, which confers high interleukin 12 production, has been identified as a marker of resistance in a cohort of HESN IDUs compared with healthy control participants and individuals with chronic hepatitis C virus infection.119 Hepatitis C virus-specific T cells secrete the antiviral and immunomodulatory cytokine interferon γ,120 A −764G→C single nucleotide polymorphism in the interferon γ promoter region, which confers increased gene expression of the Th1 response and suppresses the secretion of TNFα and interferon γ. Several studies120 suggest polymorphisms in this region of the genome are important in hepatitis C virus clearance. Particularly, a promoter region haplotype (−1117A, −854T, −627A), which is associated with reduced interleukin 10 expression, was proven to be more frequent in patients who clear primary infection.121 Transforming growth factor β suppresses NK cell activity and inhibits interferon γ and interleukin 12 production. The C allele at −509 in the promoter, which leads to reduced expression of transforming growth factor β1, has been associated with hepatitis C virus clearance in a study of Japanese patients.122

**Conclusion**

Clear precedents exist for genetically determined resistance and protection against human infectious diseases. Some epidemiological evidence exists to suggest that a small subset of individuals remain apparently uninfected with hepatitis C virus, despite very longlasting high-risk behaviours and probable repeated exposure. Some individuals repeatedly clear established infection via both innate and adaptive immune mechanisms. In combination these data suggest plausibility for both hepatitis C virus resistance and immunological protection, and that genetics probably contribute to these phenotypes.

A framework for consistent definition of the HESN phenotype using longitudinal data among high risk individuals has been proposed because plausible mechanistic subpopulations within the HESN label cannot yet be delineated. With a consistent working definition of the HESN phenotype, genetic and
immunological associations of the phenotype can be explored, and compared with other traditional hepatitis C virus outcomes, including those with chronic infection and spontaneous clears who seroconvert, some of whom might become protected from reinfection. These investigations of well characterised HESN individuals could identify the genetic correlates and host immune response characteristics of the HESN phenotype, which will inform vaccine development strategies.

Contributors
All authors contributed to the writing of this Review and agree with its content and conclusions.

Declaration of interests
We declare no competing interests.

References
1 Burgner D, Jamieson SE, Blackwell M. Genetic susceptibility to infectious diseases: big is beautiful, but will bigger be even better? Lancet Infect Dis 2006; 6: 653–63.
4 Chapman SJ, Hill AV. Human genetic susceptibility to infectious diseases: big is beautiful, but will bigger be even better? Lancet Infect Dis 2006; 6: 653–63.
20 Bianco A, Reghellini V, Domini C, et al. Metabolism of phosphatidylinositol 4-kinase IIIa-dependent PI4P is subtracted by HCV and is targeted by a 4-anilino quinazoline with antiviral activity. PLoS Pathog 2012; 8: e1002576.
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