Conference Report

Drugs in Development for Hepatitis B Targeting cccDNA

Robert G. Gish[1]

[1]Hepatitis B Foundation, Doylestown, Pennsylvania. US.

Email: rgish@robertgish.com

ABSTRACT

To completely eliminate HBV infection, the total eradication of covalently closed circular DNA (cccDNA), the mini-chromosome that provides the template for transcription of all viral mRNAs, would be required. Nuclear cccDNA accumulates in hepatocyte nuclei where it persists as a stable epigenome. Because the HBV genome is integrated into the host cell with this persistence of cccDNA a complete cure in which all cccDNA and integrated virus is eliminated is not something we currently have but is considered the ultimate aim of future therapies. The dozens of experimental CHB treatments currently being developed fall into two main categories: direct-acting antivirals that impede viral replication at a specific point and host-targeting agents that modify host cell function in a way that inhibits viral replication, including both immune modulators and agents that target other host functions. Each of these can target cccDNA directly or indirectly. Discussed here are agents currently in the clinical stage of development for CHB treatment that target cccDNA directly and those that may affect cccDNA indirectly.

Keywords: hepatitis B virus, antivirals, immunomodulators, cccDNA

Introduction

Worldwide, approximately 292 million people are chronically infected with hepatitis B virus (HBV),[1] approximately 75% of whom reside in Asia and 12% in Africa.[2] Although the prevalence of chronic hepatitis B (CHB) is much lower in Western countries, it is estimated that even in the United States as many as 2.2 million people may be chronically infected.[3] In Cuba, there are insufficient epidemiologic data to accurately estimate national CHB prevalence but since the inclusion of hepatitis B vaccine in the national immunization program in 1992 there has been a decline in the annual reported cases.[4] CHB is one of the leading causes of liver disease, cirrhosis, and hepatocellular carcinoma (HCC) worldwide.[1] In Cuba, liver cirrhosis was the ninth leading cause of death in 2018.[5] Thus, there is considerable interest in developing effective CHB therapies. The only currently approved therapies are long-term treatment with nucleos(t)ide analogues (NUCs) and a finite course of pegylated interferon-alpha (PEG-IFN-α).[6] Although these therapies have substantial benefits, decreasing the risks of HCC and liver decompensation and increasing survival,[6-8] the elimination of hepatitis B surface antigen (HBsAg) does not usually occur. HBsAg loss is seen in only 3-8% of either HBeAg-negative or HBeAg-positive CHB patients at 48-52 weeks of PEG-IFN-α therapy.[6,9,10] HBsAg loss is in the same range for patients receiving NUC therapy, only occurring in 0% to 11.8% of HBeAg-positive patients and 0.3% to 5% of HBeAg-negative patients even after multiple years of therapy.[11-14] Thus, new therapies that could substantially increase HBsAg loss and potentially allow therapy to be discontinued are of great interest.

To completely eliminate HBV infection, the total eradication of covalently closed circular DNA (cccDNA), the mini-chromosome that provides the template for transcription of all viral mRNAs,[15,16] would be required.[17] Nuclear cccDNA accumulates in hepatocyte nuclei where it persists as a stable epigenome.[16,18,19] Because the HBV genome is integrated into the host cell with this persistence of cccDNA a complete cure in which all cccDNA and integrated virus is eliminated is not something we currently have but is considered the ultimate aim of future therapies.[20]

Drugs in Development

The dozens of experimental CHB treatments currently being developed fall into two main categories: (1) direct-acting antivirals (DAAs) that impede viral replication at a specific point and (2) host-targeting agents that modify host cell function in a way that inhibits viral replication, including both immune modulators and agents that target other host functions.[21] Each of these can target cccDNA directly or indirectly. Discussed here are agents currently in the clinical stage of development for CHB treatment that target cccDNA directly and those that may affect cccDNA indirectly. A broader summary of agents currently under development for the treatment of CHB are summarized in the Table.

Direct-acting Antivirals

Antisense Oligonucleotides

Multiple antisense oligonucleotides (ASOs) are currently in development. It is thought that they may yield a decrease in cccDNA by blocking core production and capsid formation. In a duck model, use of polyethylenimine-based ASOs has been shown to result in significant reductions in viremia, intrahepatic HBV DNA, HBV RNA, and surface and core proteins.[22] This may represent an effect on cccDNA in the nucleus. It is also known that ASOs can downregulate asialoglycoprotein receptor 1 (ASGPR1) which HBV upregulates, thus blocking HBV replication by inhibiting hepatic endocytosis of HBV.

cccDNA Formation and Transcription Inhibitors

Repressing cccDNA can occur by blocking its formation, expression, or stability.[21] Cell studies have shown that there might be agents that could decrease or eliminate cccDNA. Two disubstituted sulfonamides (DSS), CCC-0975 and CCC-0346, have been shown to inhibit the formation of cccDNA from rcDNA by suppressing rcDNA deproteinization; CCC-0975 was shown to reduce cccDNA biosynthesis.[23] In another approach, cell studies have shown that activation of the lymphotoxin β receptor (LTβR) with a super-agonistic tetravalent bispecific antibody (BS1) and a

bivalent anti-LTβR monoclonal antibody (CBE11) results in non-hepatotoxic degradation of cccDNA.[24] Inhibition of cccDNA formation has also been achieved by targeting the HBV viral genome with endonucleases including meganucleases, transcription activator like effector nucleases (TALENs), zinc finger nucleases (ZFNs), and the CRISPR (clustered, regularly interspaced, short palindromic repeat)/Cas 9 genome editing tool.[25]

Core/Capsid Inhibitors

Several agents have been developed that counter HBV DNA replication by disturbing capsids or countering core particle assembly.[26] The result may be decreased cccDNA levels. Included in this class are the heteroaryldihydropyrimidines (HAPs) Bay 41-4109, HAP-1, GLS-4, HAP-18 and NVR-010-001-E2.[27,28]

HBsAg Release Inhibitors

In another approach, nucleic acid polymers (NAPs) have been studied because of their ability to inhibit protein interactions involved in viral replication. Their possible effect on cccDNA is hypothetical via reductions in HBcrAg and HBV RNA levels in the blood. In one small study of 12 patients treated with NAP HBsAg release inhibitor REP 2139-Ca followed, in responders, by peginterferon alpha-2a and/or thymosin alpha-1, there was a substantial decline in HBV DNA and HBsAg levels and HBV DNA the majority continued HBV DNA declines off treatment before subsequent rebound.[67] This agent is currently in phase 1 and 2 clinical trials in combination with Peg-IFN and TDF.

Reverse Transcriptase Inhibitors

Because nucleos(t)ide analogue reverse transcriptase (RT) inhibitors (NUCs) do not directly suppress cccDNA viral transcription or translation they are not considered major inhibitors of cccDNA, although a 1-2 log reduction in cccDNA has been seen in some studies in liver tissue where cccDNA levels could be measured. Clevudine, a NUC approved for HBV treatment in South Korea and the Philippines,[29] is no longer used as a solo agent because of adverse effects (myopathy) and drug resistance but it is used in lower doses in combination with adefovir[30-32] and

a new form of clevudine that it is thought may affect cccDNA levels via a new mechanism is entering phase I trials.

Although Lai and colleagues have reported that prolonged NUC treatment (median 126 months), markedly reduced cccDNA, it was also shown that, although reduced, serum HBsAg levels remained detectable in 42 of 43 patients.[33] Long-term treatment with NUCs can be associated with drug toxicity and drug resistance.[34,35] Therefore, there is a need to identify compounds that can lead to eradication of the virus.

RNA Interference Therapies

RNA interference (RNAi) therapies directly target hepatitis B virus mRNA transcripts and have been shown to do so with high specificity. They reduce HBsAg and HBcAg production through the use of small, non-coding RNAs that regulate the expression of genetic information.[36] This may restore host immunity[37] and decrease cccDNA replenishment.

Host-Targeting Agents

Included in this category are a broad variety of agents that modify various aspects of host cell function in ways that inhibit viral replication. These agents can be broadly subdivided into immune modulators and agents that target other host functions.[21] Such host-targeting agents may be able to boost both innate and adaptive immunity, thus helping to clear HBV-infected hepatocytes.

Engineered T cells

Engineering T cells is designed to boost the attraction of T-cell receptors for specific antigens. Preclinical studies have been promising. In HBV transgenic mice, CD8(+) T cells engineered to express HBV-specific chimeric antigen receptors (CARs) were shown to recognize multiple HBV subtypes and to be able to engraft and expand, localizing to the liver and quickly decreasing HBV replication.[38] In an important recent study by Protzer and colleagues, in HBV-infected hepatoma cells, co-culture with T cells engineered to express high-affinity T-cell receptors specific for HBV core or envelope proteins led to undetectable levels of cccDNA and viral antigens.[39] In HBV-infected humanized mice, adoptive transfer of T-cell receptor-grafted T cells led to clearance of

HBV-infected hepatocytes and major decreases in HBV viral load and cccDNA.[39] A clinical trial of therapy with engineered T cells in patients with HBV-associated HCC is now planned. Although promising, the very high cost of this individualized therapy and the serious adverse effects seen in other diseases where it has been studied will no doubt limit its use, especially in the developing world.

Entry Inhibitors

Entry inhibitors could block HBV entry into hepatocytes before cccDNA is even formed.[40] They work by either blocking HBV binding to the cell receptor(s) or HBV attachment to hepatocytes.[41] Myrcludex B is a synthetic N-myristoylated peptide that competitively attaches to the sodium taurocholate cotransporting polypeptide (NTCP) receptor, thus preventing HBV from entering hepatocytes.[42] In one study it was shown that myrcludex B prevented intrahepatic virus spreading in humanized mice and hindered amplification of intrahepatic cccDNA by blocking the conversion of rcDNA to cccDNA.[42]

Immunomodulatory Agents

Much work has been done on approaches to altering the immune response to HBV in order to restore effective antiviral immune responses and many immunomodulatory agents are currently being assessed, including therapeutic vaccines, engineered T cells, toll-like receptor agonists, immune checkpoint inhibitors, and others.

Toll-like Receptor Agonists

It is known that HBV reduces the toll-like receptor (TLR) antiviral activity of liver cells.[43] These receptors are key players in immune responses because they sense pathogens and boost inflammatory cytokine release and the adaptive immune responses that follow.[44] TLR7 stimulation mediates an endogenous type I interferon response which is key for developing an effective immunity against HBV.[45] In preclinical studies, activation of intrahepatic innate immune responses with TLRs 3/7/8/9 or STING agonists has been shown to suppress HBV.[46-50] Multiple doses of the TLR7 agonist GS-9620 given to chimpanzees were shown to result in significant reductions in

viral load, HBsAg, and HBeAg[51] but, after phase 1 trials did not show reductions in HBsAg levels or HBV DNA with monotherapy[52], its usefulness in combination with TDF is now being studied in phase 2 trials.[44]

Other Immune Modulators and Associated Therapies

Current studies are assessing the immune restoration and vaccine adjuvant effects of certain cytokines and cytokine receptor agonists which it is thought might eliminate cccDNA. SB 9200 is a novel therapy that may not only have direct antiviral properties but also have the ability to prompt endogenous IFN-mediated immune responses in HBV-infected cells. The latter is accomplished by activation of retinoic acid-inducible gene 1 (RIG-I) and nucleotide-binding oligomerization domain-containing protein 2 (NOD2). In woodchucks, daily dosing of SB 9200 for 12 weeks resulted in substantial reductions in viral load and in surface antigen, the latter thought to result from suppression of cccDNA synthesis or transcription.[53]

Because the effectiveness of some immune modulators may be reduced by high antigen levels[54-56] the CRISPR (clustered, regularly interspaced, short palindromic repeat)/Cas9 genome editing tool that has been called a type of molecular scissors has been studied as an approach to removing HBV cccDNA, both in cell studies [57,58] and using a mouse model.[59-61] Although it is hoped that this technique could ultimately be used to directly remove cccDNA, thus improving the effectiveness of certain immune modulators,[44] recent studies showing that it may damage DNA located far from the target DNA[62,63] has led researchers to urge additional cautionary measures as these therapies are developed.

Conclusion

Although it is clear that current HBV therapies reduce progression to chronic liver disease and its sequelae, many different treatments with multiple targets, including both virologic approaches and host immune approaches, are being studied to achieve the ultimate goal: elimination of cccDNA.[64-66] Almost all of these approaches are in pre-clinical or phase 1 or 2 trials. Until a true sterilizing cure can be achieved in which all cccDNA and integrated virus are removed, the approaches that work the best with CHB may be a combination of effective antiviral therapies and host-targeting

agents. Research advances have shown that such a combined approach may lead to what has been termed a functional cure for CHB in the not distant future.

References

1. Polaris Observatory Collaborators. Global prevalence, treatment, and prevention of hepatitis B virus infection in 2016: a modelling study. Lancet Gastroenterol Hepatol. 2018;3:383-403.
2. Gust ID. Epidemiology of hepatitis B infection in the Western Pacific and South East Asia. Gut 1996;38 Suppl 2:S18-23.
3. Kowdley KV, Wang CC, Welch S, et al. Prevalence of chronic hepatitis B among foreign-born persons living in the United States by country of origin. Hepatology 2012;56:422-433.
4. Marlen Ivon CF, Zaily DG, Conde-Eduardo Leda Patricia DS, et al. Current Condition of Chronic Hepatitis B Virus Infection in Cuban Adults. Curr Ther Res Clin Exp 2017;85:15-19.
5. Dirección de Registros Médicos y Estadísticas de Salud. Anuario estadistico de salud La Habana: MINSAP. 2018; Available at: http://www.sld.cu/sitios/dne/. Accessed October 15, 2019.
6. Lok AS, McMahon BJ. Chronic hepatitis B: Update 2009. Hepatology 2009;50:661-662.
7. Rijckborst V, ter Borg MJ, Cakaloglu Y, et al. A randomized trial of peginterferon alpha-2a with or without ribavirin for HBeAg-negative chronic hepatitis B. Am J Gastroenterol 2010;105:1762-1769.
8. Sung JJ, Tsoi KK, Wong VW, et al. Meta-analysis: Treatment of hepatitis B infection reduces risk of hepatocellular carcinoma. Aliment Pharmacol Ther 2008;28:1067-1077.
9. Buster EHCJ, Janssen HLA. Antiviral treatment for chronic hepatitis B virus infection - immune modulation or viral suppression. Neth J Med 2006;64:175-185.
10. Lampertico P. The royal wedding in chronic hepatitis B: The haves and the have-nots for the combination of pegylated interferon and nucleos(t)ide therapy. Hepatology 2015;61:1459-1461.

11. Chang TT, Lai CL, Kew Yoon S, et al. Entecavir treatment for up to 5 years in patients with hepatitis B e antigen-positive chronic hepatitis B. Hepatology 2010;51:422-430.
12. Buti M, Tsai N, Petersen J, et al. Seven-year efficacy and safety of treatment with tenofovir disoproxil fumarate for chronic hepatitis B virus infection. Dig Dis Sci 2015;60:1457-1464.
13. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, et al. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B for up to 5 years. Gastroenterology 2006;131:1743-1751.
14. Pan CQ, Tong M, Kowdley KV, et al. High rates of viral suppression after long-term entecavir treatment of Asian patients with hepatitis B e antigen-positive chronic hepatitis B. Clin Gastroenterol Hepatol 2012;10:1047-1050 e1041.
15. Warner N, Locarnini S. Replication of hepatitis B virus. In: Boyer TD, Manns MP, Sanyal AJ, eds. *Zakim and Boyer's Hepatology: A Textbook of Liver Disease*. 6 ed. Philadelphia: Elsevier; 2012.
16. Belloni L, Pollicino T, De Nicola F, et al. Nuclear HBx binds the HBV minichromosome and modifies the epigenetic regulation of cccDNA function. Proc Natl Acad Sci U S A 2009;106:19975-19979.
17. Ohno M, Otsuka M, Kishikawa T, et al. Novel therapeutic approaches for hepatitis B virus covalently closed circular DNA. World J Gastroenterol 2015;21:7084-7088.
18. Levrero M, Pollicino T, Petersen J, et al. Control of cccDNA function in hepatitis B virus infection. J Hepatol 2009;51:581-592.
19. Zhu Y, Yamamoto T, Cullen J, et al. Kinetics of hepadnavirus loss from the liver during inhibition of viral DNA synthesis. J Virol 2001;75:311-322.
20. Gish RG, Given BD, Lai CL, et al. Chronic hepatitis B: Virology, natural history, current management and a glimpse at future opportunities. Antiviral Res 2015;121:47-58.
21. Block TM, Rawat S, Brosgart CL. Chronic hepatitis B: A wave of new therapies on the horizon. Antiviral Res 2015;121:69-81.
22. Robaczewska M, Guerret S, Remy JS, et al. Inhibition of hepadnaviral replication by polyethylenimine-based intravenous delivery of antisense phosphodiester oligodeoxynucleotides to the liver. Gene Ther 2001;8:874-881.

23. Cai D, Mills C, Yu W, et al. Identification of disubstituted sulfonamide compounds as specific inhibitors of hepatitis B virus covalently closed circular DNA formation. Antimicrob Agents Chemother 2012;56:4277-4288.
24. Lucifora J, Xia Y, Reisinger F, et al. Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. Science 2014;343:1221-1228.
25. Cox DB, Platt RJ, Zhang F. Therapeutic genome editing: prospects and challenges. Nat Med 2015;21:121-131.
26. Wu G, Liu B, Zhang Y, et al. Preclinical characterization of GLS4, an inhibitor of hepatitis B virus core particle assembly. Antimicrob Agents Chemother 2013;57:5344-5354.
27. Deres K, Schroder CH, Paessens A, et al. Inhibition of hepatitis B virus replication by drug-induced depletion of nucleocapsids. Science 2003;299:893-896.
28. Cole AG. Modulators of HBV capsid assembly as an approach to treating hepatitis B virus infection. Curr Opin Pharmacol 2016;30:131-137.
29. Jang JH, Kim JW, Jeong SH, et al. Clevudine for chronic hepatitis B: antiviral response, predictors of response, and development of myopathy. J Viral Hepat 2011;18:84-90.
30. Seok JI, Lee DK, Lee CH, et al. Long-term therapy with clevudine for chronic hepatitis B can be associated with myopathy characterized by depletion of mitochondrial DNA. Hepatology 2009;49:2080-2086.
31. Kim SB, Song IH, Kim YM, et al. Long-term treatment outcomes of clevudine in antiviral-naive patients with chronic hepatitis B. World J Gastroenterol 2012;18:6943-6950.
32. Tak WY, Yang JM, Kim BI, et al. A randomized, open-label study comparing low-dose clevudine plus adefovir combination therapy with clevudine monotherapy in naive chronic hepatitis B patients. Hepatol Int 2014;8:375-381.
33. Lai CL, Wong D, Ip P, et al. Reduction of covalently closed circular DNA with long-term nucleos(t)ide analogue treatment in chronic hepatitis B. J Hepatol 2017;66:275-281.
34. Scaglione SJ, Lok AS. Effectiveness of hepatitis B treatment in clinical practice. Gastroenterology 2012;142:1360-1368 e1361.
35. Zoulim F, Locarnini S. Hepatitis B virus resistance to nucleos(t)ide analogues. Gastroenterology 2009;137:1593-1608 e1591-1592.

36. Carthew RW, Sontheimer EJ. Origins and mechanisms of miRNAs and siRNAs. Cell 2009;136:642-655.
37. Schluep T, Lickliter J, Hamilton J, et al. Safety, tolerability and pharmacokinetics of ARC-520 Injection, an RNA interference-based therapeutic for the treatment of chronic hepatitis B virus infection, in healthy volunteers. Clin Pharmacol Drug Dev 2016:[Epub ahead of print].
38. Krebs K, Bottinger N, Huang LR, et al. T cells expressing a chimeric antigen receptor that binds hepatitis B virus envelope proteins control virus replication in mice. Gastroenterology 2013;145:456-465.
39. Wisskirchen K, Kah J, Malo A, et al. T cell receptor grafting allows virological control of Hepatitis B virus infection. J Clin Invest 2019;129:2932-2945.
40. Kaneko M, Watashi K, Kamisuki S, et al. A novel tricyclic polyketide, vanitaracin A, specifically inhibits the entry of hepatitis B and D viruses by targeting sodium taurocholate cotransporting polypeptide. J Virol 2015;89:11945-11953.
41. Ye X, Zhou M, He Y, et al. Efficient inhibition of hepatitis B virus infection by a preS1-binding peptide. Sci Rep 2016;6:29391.
42. Volz T, Allweiss L, Ben MM, et al. The entry inhibitor Myrcludex-B efficiently blocks intrahepatic virus spreading in humanized mice previously infected with hepatitis B virus. J Hepatol 2013;58:861-867.
43. Wu J, Meng Z, Jiang M, et al. Hepatitis B virus suppresses toll-like receptor-mediated innate immune responses in murine parenchymal and nonparenchymal liver cells. Hepatology 2009;49:1132-1140.
44. Pham EA, Perumpail RB, Fram BJ, et al. Future therapy for hepatitis B virus: role of immunomodulators. Curr Hepatol Rep 2016;15:237-244.
45. Funk E, Kottilil S, Gilliam B, et al. Tickling the TLR7 to cure viral hepatitis. J Transl Med 2014;12:129.
46. Chang J, Guo JT. Treatment of chronic hepatitis B with pattern recognition receptor agonists: Current status and potential for a cure. Antiviral Res 2015;121:152-159.

47. Menne S, Tumas DB, Liu KH, et al. Sustained efficacy and seroconversion with the Toll-like receptor 7 agonist GS-9620 in the Woodchuck model of chronic hepatitis B. J Hepatol 2015;62:1237-1245.
48. Wu J, Huang S, Zhao X, et al. Poly(I:C) treatment leads to interferon-dependent clearance of hepatitis B virus in a hydrodynamic injection mouse model. J Virol 2014;88:10421-10431.
49. Jo J, Tan AT, Ussher JE, et al. Toll-like receptor 8 agonist and bacteria trigger potent activation of innate immune cells in human liver. PLoS Pathog 2014;10:e1004210.
50. Huang LR, Wohlleber D, Reisinger F, et al. Intrahepatic myeloid-cell aggregates enable local proliferation of CD8(+) T cells and successful immunotherapy against chronic viral liver infection. Nat Immunol 2013;14:574-583.
51. Lanford RE, Guerra B, Chavez D, et al. GS-9620, an oral agonist of Toll-like receptor-7, induces prolonged suppression of hepatitis B virus in chronically infected chimpanzees. Gastroenterology 2013;144:1508-1517, 1517 e1501-1510.
52. Gane EJ, Lim YS, Gordon SC, et al. The oral toll-like receptor-7 agonist GS-9620 in patients with chronic hepatitis B virus infection. J Hepatol 2015;63:320-328.
53. Korolowicz KE, Iyer RP, Czerwinski S, et al. Antiviral efficacy and host innate immunity associated with SB 9200 treatment in the woodchuck model of chronic hepatitis B. PLoS One 2016;11:e0161313.
54. Barnes E. Therapeutic vaccines in HBV: lessons from HCV. Med Microbiol Immunol 2015;204:79-86.
55. Weng M, Zeng WZ, Wu XL, et al. Quantification of serum hepatitis B surface antigen in predicting the response of pegylated interferon alfa-2a in HBeAg-positive chronic hepatitis B with prior lamivudine exposure. Virol J 2013;10:277.
56. Reignat S, Webster GJ, Brown D, et al. Escaping high viral load exhaustion: CD8 cells with altered tetramer binding in chronic hepatitis B virus infection. J Exp Med 2002;195:1089-1101.
57. Seeger C, Sohn JA. Targeting hepatitis B virus with CRISPR/Cas9. Mol Ther Nucleic Acids 2014;3:e216.

58. Karimova M, Beschorner N, Dammermann W, et al. CRISPR/Cas9 nickase-mediated disruption of hepatitis B virus open reading frame S and X. Sci Rep 2015;5:13734.
59. Dong C, Qu L, Wang H, et al. Targeting hepatitis B virus cccDNA by CRISPR/Cas9 nuclease efficiently inhibits viral replication. Antiviral Res 2015;118:110-117.
60. Lin SR, Yang HC, Kuo YT, et al. The CRISPR/Cas9 system facilitates clearance of the intrahepatic HBV templates in vivo. Mol Ther Nucleic Acids 2014;3:e186.
61. White MK, Hu W, Khalili K. The CRISPR/Cas9 genome editing methodology as a weapon against human viruses. Discov Med 2015;19:255-262.
62. Haapaniemi E, Botla S, Persson J, et al. CRISPR-Cas9 genome editing induces a p53-mediated DNA damage response. Nat Med 2018;24:927-930.
63. Ihry RJ, Worringer KA, Salick MR, et al. p53 inhibits CRISPR-Cas9 engineering in human pluripotent stem cells. Nat Med 2018;24:939-946.
64. Lin CL, Yang HC, Kao JH. Hepatitis B virus: new therapeutic perspectives. Liver Int 2016;36 Suppl 1:85-92.
65. Coffin CS, Lee SS. New paradigms in hepatitis B management: only diamonds are forever. Br Med Bull 2015;116:79-91.
66. Petersen J, Thompson AJ, Levrero M. Aiming for cure in HBV and HDV infection. J Hepatol 2016;65:835-848.
67. Al-Mahtab M, Bazinet M, Vaillant A, et al. Safety and Efficacy of Nucleic Acid Polymers in Monotherapy and Combined with Immunotherapy in Treatment-Naive Bangladeshi Patients with HBeAg+ Chronic Hepatitis B Infection. PLoS One. 2016;11:e0156667.