SCIENCE BEHIND THE STUDY
Elizabeth G. Phimister, Ph.D., *Editor*

Combination Therapy for Chronic HBV Infection

Harry L.A. Janssen, M.D., Ph.D., and Milan J. Sonneveld, M.D., Ph.D.

In this issue of the *Journal*, Hou et al.[1] describe the efficacy and safety of 48 weeks of treatment with xalnesiran, an RNA interference therapeutic, alone or in combination with an immunomodulator in patients with chronic hepatitis B virus (HBV) infection already treated with a nucleoside or nucleotide analogue. The immunomodulators used in the trial were pegylated interferon alfa-2a and ruzotolimod. The primary end point, loss of the hepatitis B surface antigen (HBsAg) at 24 weeks after the end of the treatment period, was observed in none of the patients treated with nucleoside or nucleotide analogues alone, 3 to 7% of those treated with xalnesiran alone, 12% of those treated with xalnesiran and ruzotolimod, and 23% of those treated with xalnesiran and pegylated interferon alfa-2a.

There was an erosion of the suppressive effect on HBsAg after withdrawal of xalnesiran alone or combined with an immune modulator. These results, however, herald a new era for combination therapy to treat chronic HBV infection and raise questions about how and when to assess response and how to balance the probability of response with the likelihood of durable clinical benefit and risk of side effects.

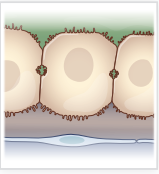
WHAT IS THE GLOBAL BURDEN OF CHRONIC HBV INFECTION?

Chronic HBV infection affects more than 3% of the world population[2] and is responsible for approximately 1.1 million deaths annually. Premature death in patients is predominantly due to complications of cirrhosis, portal hypertension, and hepatocellular carcinoma (Fig. 1).[3] Case finding and treatment of patients at risk for liver-related complications is therefore essential to mitigate this major global health threat.

WHAT IS FUNCTIONAL CURE OF CHRONIC HBV INFECTION?

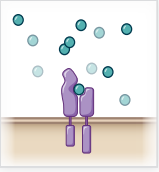
HBV uses the cell-surface sodium taurocholate cotransporting polypeptide (NTCP) to enter hepatocytes (see Key Concepts), after which its genome becomes a covalently closed circular DNA (cccDNA); a reservoir of HBV cccDNAs is then

Key Concepts



Hepatocyte

A primary cell of the liver. Hepatocytes make up 70 to 80% of the liver's mass and are involved in many of the liver's key functions, including detoxification and the metabolism of carbohydrates and lipids. In addition, they are involved in the production of proteins, such as albumin, clotting factors and various complement factors, and bile. They are infected by hepatotropic viruses including hepatitis A, B, C, D, and E viruses. The liver has a remarkable ability to regenerate because hepatocytes can reenter the cell cycle from a quiescent phase. Nevertheless, in the context of chronic liver disease, such as chronic hepatitis B virus infection, they are slowly displaced by fibrous tissue, eventually leading to cirrhosis, liver failure, and liver cancer.



Interferon-α

A cytokine and type I interferon synthesized primarily by certain types of hematopoietic cells after the stimulation of pattern-recognition receptors such as the toll-like receptors and RIG-I–like receptors. These receptors are typically activated by viral DNA and other pathogen- associated molecular patterns. Through binding the interferon-α/ß receptor on the surface of dendritic cells, type I interferons activate downstream processes that result in the induction of interferon-stimulated genes, which have myriad antiviral effects. In addition, type I interferons stimulate innate and adaptive cellular immune responses by activating B cells, T cells, and natural killer cells. The addition of a polyethylene glycol group to interferon alfa (resulting in pegylated interferon alfa) prolongs its half-life and permits less frequent dose administration than that with interferon alfa.

Toll-like receptor

A receptor protein expressed by macrophages and other cells that detect and respond to pathogens. (Toll-like receptors are also known as pattern-recognition receptors.) Certain toll-like receptors (e.g., TLR7 and TLR8) can recognize molecules broadly shared by pathogens (the so-called pathogen-associated molecular patterns, or PAMPs) that are distinct from normal host molecules. Certain toll-like receptors (e.g., TLR7) play a role in the activation of autoreactive B cells and dendritic cells by self-antigens. Activation of toll-like receptors (which are expressed on innate immune cells and other cell types) by PAMPs initiates an immune response involving the production of type I interferons and other cytokines. Agonists of toll-like receptors are under investigation for the treatment of viral infections and cancer.

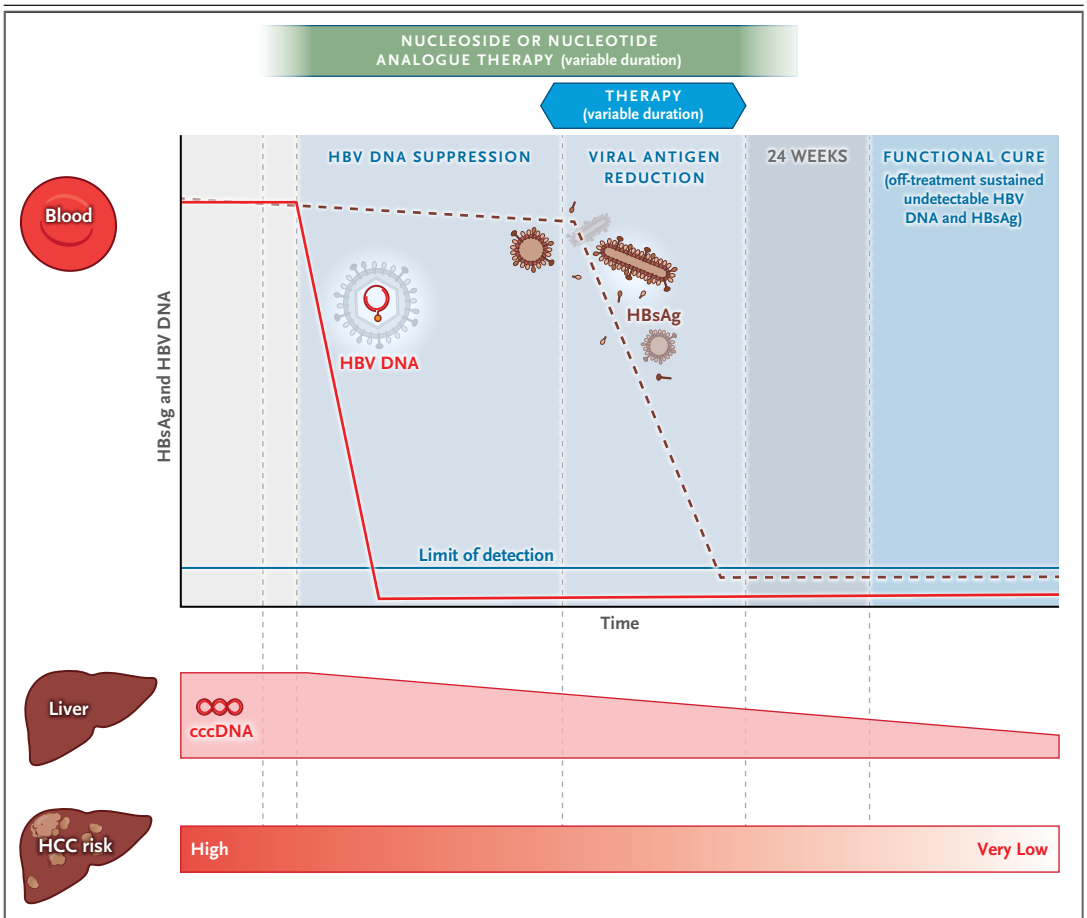


Figure 1. New Treatment Paradigms in Chronic Hepatitis B Virus (HBV) Infection — From Viral Suppression to Functional Cure.

Patients with high HBV DNA levels in peripheral blood and signs of liver inflammation have an indication for antiviral therapy. At this disease stage, intrahepatic covalently closed circular DNA (cccDNA) levels are also high, as is the risk of hepatocellular carcinoma (HCC). Nucleoside and nucleotide analogues effectively suppress HBV DNA levels but have little effect on viral antigen production. Concomitant combination with or addition of other drugs with different modes of action, such as direct interference with viral antigen production and immune modulation, can help decrease hepatitis B surface antigen (HBsAg) levels and result in functional cure. HBV DNA suppression alone may reduce the risk of HCC as compared with no treatment, but achievement of functional cure is associated with the lowest residual risk of HCC among patients without another coexisting liver disease or established cirrhosis.

established in the cell nucleus. The cccDNA provides the template from which HBV RNAs are synthesized — these are vital for both viral replication and viral protein production, including HBsAg (Fig. 2). Importantly, viral DNA can also be integrated into host DNA, thereby becoming an independent source of HBsAg production.[4] Because eradication of the cccDNA reservoir cannot be achieved with current treatments (except liver transplantation), a commonly used end point is a "functional cure," defined as off-treatment sustained undetectable HBV DNA and HBsAg. Functional cure virtually eliminates the risk of liver fibrosis progression, in the absence of other coexisting liver disease, and profoundly reduces the risk of hepatocellular carcinoma among patients without established cirrhosis. Other advantages of a functional cure include avoidance of the long-term side effects and costs of medication and continued stigma.

If a functional cure cannot be achieved, suppression of HBV DNA levels with persistently posi-

tive HBsAg may be a second-best outcome. However, this outcome is associated with an excess risk of hepatocellular carcinoma as compared with functional cure.

WHAT IS THE STANDARD OF CARE?

Nucleoside or nucleotide analogues currently form the backbone of therapy for most patients with chronic HBV infection who have access to treatment. They disrupt synthesis of HBV DNA by competitively binding the HBV polymerase (the viral enzyme that generates copies of HBV DNA) and thus suppress HBV DNA levels in the serum; they achieve complete suppression of HBV DNA in peripheral blood in nearly all adherent patients. However, treatment must be continued on a long-term basis. Nucleoside and nucleotide analogues do not affect other steps in the viral replication cycle and therefore do not eliminate viral antigens, including HBsAg, so they hardly ever provide functional cure.[4] Although treatment with nucleoside or nucleotide analogues is associated with a decrease in the severity of liver fibrosis and reduction of liver-related complications, the risk of hepatocellular carcinoma persists at the population level because functional cure is not achieved in most patients.[5]

HOW DOES XALNESIRAN COUNTER HBV?

Xalnesiran is a small interfering RNA (siRNA) that targets the conserved S-region (encoding the surface antigen) of the HBV genome. After its delivery to the hepatocyte, which is facilitated by the conjugation of an *N*-acetyl-D-galactosamine (GalNAc) to one end of the double-stranded RNA, the latter is loaded into a protein complex called the RNA-induced silencing complex (RISC). After the so-called passenger strand is discarded, the exposed guide strand pairs with the target messenger RNA, which is then sliced and degraded by an enzymatic component of RISC. The result is the prolonged suppression of myriad RNA transcripts synthesized from both cccDNA and integrated HBV DNA (Fig. 1). In a phase 1 trial, treatment with four monthly doses of xalnesiran led to marked reductions in serum HBsAg levels that were sustained for up to a year of subsequent follow-up. Given the rapid decrease in HBsAg levels observed with short-term siRNA treatment, high rates of HBsAg clearance were anticipated with prolonged treatment, but studies of longer-term siRNA monotherapy showed a plateauing of the decrease in HBsAg levels after several months.[6] Functional cure is therefore rarely observed with siRNA monotherapy.

AND INTERFERON ALFA AND RUZOTOLIMOD?

Persistence of chronic hepatitis B is attributable to dysfunctional innate and adaptive immune responses to HBV. Therefore, reinvigoration of the immune response could be key to increasing the likelihood of functional cure.

Interferon alfa has effects on both innate and adaptive immunity and has antiviral effects mediated by induction of interferon-stimulated genes (Fig. 1), the downstream effects of which can result in the eradication of cccDNA from hepatocytes as well as suppression of HBV replication at different steps of the viral life cycle.[7] Pegylated interferon alfa is licensed for the treatment of chronic HBV infection for up to 48 weeks and results in a significantly higher incidence of functional cure than nucleoside or nucleotide analogue therapy.[8] However, the clinical application of pegylated interferon alfa is limited by side effects, such as flulike symptoms, fatigue, weight loss, mood changes, and cytopenia, and is therefore infrequently used as a monotherapy.

Ruzotolimod is an orally administered agonist of toll-like receptor 7 (TLR7) that is under investigation. Toll-like receptors are members of the family of pattern-recognition receptors that have an important role in the innate immune system through pathogen recognition. TLR7 stimulation has been shown to induce the release of cytokines, including type I interferons, that activate natural killer cells, T cells, and intrahepatic dendritic cells. In a phase 1 study,[9] ruzotolimod treatment was associated with mild systemic symptoms associated with induction of the immune system, including an influenza-like illness, reminiscent of therapy with interferons. Short-term treatment with TLR7 agonists was associated with increased cytokine production but limited decrease in HBV DNA and HBsAg levels.[9,10]

WHY COMBINE AN ANTIVIRAL WITH AN IMMUNOMODULATOR?

Combining drugs with different target engagement can provide a synergistic effect on viral suppression and immune control, leading to functional cure. This concept is supported by several

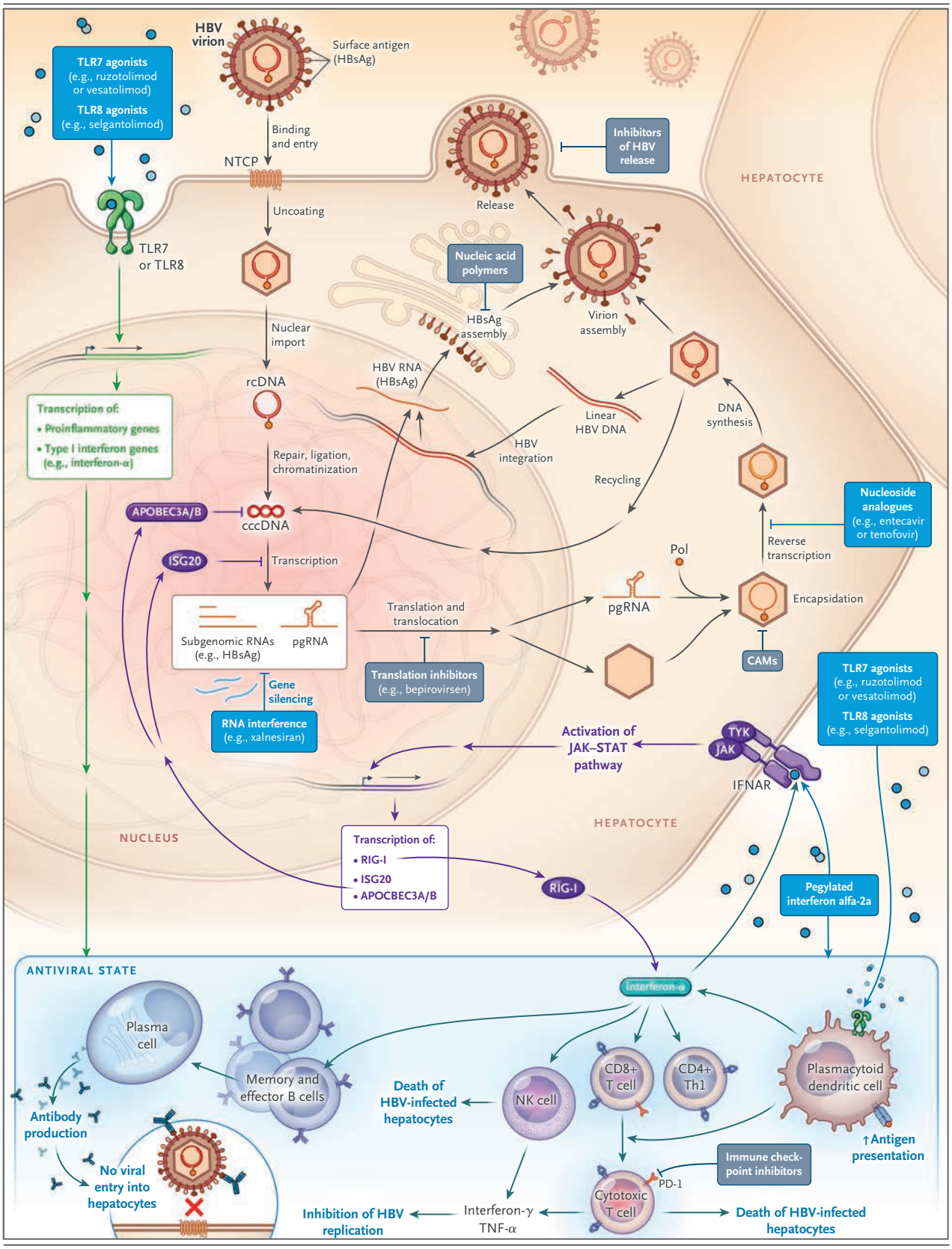


Figure 2 (facing page). Treatment Strategies for Chronic HBV Infection.

The major steps of the HBV life cycle and the antiviral and immunomodulatory approaches are depicted. More specifically, nucleoside or nucleotide analogues primarily inhibit polymerase (Pol) activity to suppress HBV DNA production. Xalnesiran is an *N*-acetyl-D-galactosamine conjugated small interfering RNA targeting the S conserved region of the HBV genome and silences viral transcripts synthesized from cccDNA and integrated HBV DNA (inhibition of transcripts synthesized from integrated HBV DNA is not shown). Ruzotolimod is a toll-like receptor 7 (TLR7) agonist that induces an antiviral state mainly by the production of type I interferons by immune cells, especially plasmacytoid dendritic cells. Secretion of interferon-α through induction by ruzotolimod or by means of administration of pegylated interferon alfa-2a exerts antiviral activity through binding to the cell-surface receptors on hepatocytes to activate the Janus kinase (JAK)–signal transducer and activator of transcription (JAK–STAT) pathway, inducing the expression of interferon-stimulated genes (ISGs), the ultimate antiviral effectors inhibiting various steps of the viral replication cycle. Among these ISGs, APOBEC3A/B and ISG20 mediate the decay of cccDNA. RIG-I is an intracellular pattern-recognition receptor that after stimulation also activates pathways resulting in induction of proinflammatory cytokines including interferon. Interferon-α has many effects on chronic HBV infection. It activates dendritic cells, promoting their presentation of antigens, in addition to CD4 and CD8 T lymphocytes. It increases the number of memory and effector B cells. Its activation of natural killer (NK) cells and cytotoxic T cells results in destruction of HBV-infected hepatocytes and in the production of cytokines (e.g., interferon-γ and tumor necrosis factor α [TNF-α]) that suppress HBV replication in hepatocytes. CAMs denotes capsid assembly modulators, IFNAR interferon-α receptor, NTCP sodium taurocholate cotransporting polypeptide, PD-1 programmed death 1, pgRNA pregenomic RNA, rcDNA relaxed circular DNA, Th1 type 1 helper T cell, TLR8 toll-like receptor 8, and TYK tyrosine kinase.

trials of combination treatment for chronic HBV infection that showed that combining nucleoside or nucleotide analogues with pegylated interferon alfa resulted in greater decreases in on-treatment HBV DNA and viral-antigen levels and a potentially higher incidence of functional cure than with either treatment alone.[11] Furthermore, the addition of pegylated interferon alfa to long-term therapy with nucleoside or nucleotide analogues in randomized trials resulted in a higher incidence of functional cure and greater decrease in HBsAg levels, albeit to a modest degree.[12,13] Because immune exhaustion that is related to a high burden of viral antigens appears to have an important role in the chronicity of HBV infection, reducing the burden of viral antigens with RNA interference could help to restore the immune response, potentially facilitating viral clearance after immunomodulatory therapy.[14]

What's Next?

Further data are needed on the durability of the effect achieved with new agents that directly interfere with HBsAg production. The results reported by Hou et al. indicate a risk of relapse, undermining the choice of functional cure as an end point for these agents, at least when assessed relatively early after the withdrawal of therapy. Future trials could evaluate whether alternative treatment regimens, such as drugs with different target engagement, could increase the likelihood of response. Hou et al. observed that functional cure was achieved only in patients with a baseline HBsAg level of less than 1000 IU per milliliter. Although a substantial proportion of patients have similarly low HBsAg levels, patients with higher HBsAg levels have the greatest risk of adverse liver-related outcomes and thus have the most to gain from new therapies; developing treatment strategies that achieve a functional cure in such patients is therefore a major goal. An additional challenge will be to identify the patient populations most likely to have a response to specific treatments.

Disclosure forms provided by the authors are available with the full text of this editorial at NEJM.org.

We thank A. Boonstra, Ph.D., Erasmus MC, Rotterdam, the Netherlands, for his advice and for his help in drafting earlier versions of the figures.

From the Department of Gastroenterology and Hepatology, Erasmus MC University Medical Center, Rotterdam, the Netherlands (H.L.A.J., M.J.S.); and the Toronto Centre for Liver Disease, Toronto General Hospital, University Health Network, Toronto (H.L.A.J.).

This editorial was updated on December 5, 2024, at NEJM.org.

1. Hou J, Zhang W, Xie Q, et al. Xalnesiran with or without an immunomodulator in chronic hepatitis B. N Engl J Med 2024;391:2098-109.
2. Polaris Observatory Collaborators. Global prevalence, cascade of care, and prophylaxis coverage of hepatitis B in 2022: a modelling study. Lancet Gastroenterol Hepatol 2023;8:879-907.
3. World Health Organization. Global hepatitis report 2024: action for access in low- and middle-income countries. April 9, 2024 (https://www.who.int/publications/i/item/9789240091672).
4. Dusheiko G, Agarwal K, Maini MK. New approaches to chronic hepatitis B. N Engl J Med 2023;388:55-69.
5. Yip TC-F, Wong GL-H, Chan HL-Y, et al. HBsAg seroclearance further reduces hepatocellular carcinoma risk after complete viral suppression with nucleos(t)ide analogues. J Hepatol 2019;70:361-70.
6. Gane EJ, Kim W, Lim TH, et al. First-in-human randomized

study of RNAi therapeutic RG6346 for chronic hepatitis B virus infection. J Hepatol 2023;79:1139-49.
7. Gehring AJ, Protzer U. Targeting innate and adaptive immune responses to cure chronic HBV infection. Gastroenterology 2019;156:325-37.
8. Lau GK, Piratvisuth T, Luo KX, et al. Peginterferon alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 2005;352:2682-95.
9. Yuen M-F, Balabanska R, Cottreel E, et al. TLR7 agonist RO7020531 versus placebo in healthy volunteers and patients with chronic hepatitis B virus infection: a randomised, observer-blind, placebo-controlled, phase 1 trial. Lancet Infect Dis 2023;23:496-507.
10. Janssen HLA, Brunetto MR, Kim YJ, et al. Safety, efficacy and pharmacodynamics of vesatolimod (GS-9620) in virally suppressed patients with chronic hepatitis B. J Hepatol 2018;68:431-40.
11. Marcellin P, Ahn SH, Ma X, et al. Combination of tenofovir disoproxil fumarate and peginterferon α-2a increases loss of hepatitis B surface antigen in patients with chronic hepatitis B. Gastroenterology 2016;150(1):134-144.e10.
12. Farag MS, van Campenhout MJH, Sonneveld MJ, et al. Addition of PEG-interferon to long-term nucleos(t)ide analogue therapy enhances HBsAg decline and clearance in HBeAg-negative chronic hepatitis B: multicentre randomized trial (PAS Study). J Viral Hepat 2024;31:197-207.
13. Bourlière M, Rabiega P, Ganne-Carrie N, et al. Effect on HBs antigen clearance of addition of pegylated interferon alfa-2a to nucleos(t)ide analogue therapy versus nucleos(t)ide analogue therapy alone in patients with HBe antigen-negative chronic hepatitis B and sustained undetectable plasma hepatitis B virus DNA: a randomised, controlled, open-label trial. Lancet Gastroenterol Hepatol 2017;2:177-88.
14. Yang S, Zeng W, Zhang J, Lu F, Chang J, Guo J-T. Restoration of a functional antiviral immune response to chronic HBV infection by reducing viral antigen load: if not sufficient, is it necessary? Emerg Microbes Infect 2021;10:1545-54.

DOI: 10.1056/NEJMe2410543

Cell-free DNA Screening and Maternal Cancer

Neeta L. Vora, M.D.

In 2013, I, along with other investigators, described a healthy woman with an uncomplicated pregnancy who was referred to maternal fetal medicine and genetic counseling because she received positive results for trisomy 13 and monosomy 18 through a screening assay of cell-free DNA (cfDNA) obtained from a blood sample.[1] These results were difficult to explain because the ultrasound showed a structurally normal fetus. An amniocentesis was performed to obtain fetal cells for genetic testing. The results of the fetal karyotype and chromosomal microarray analysis were normal. The pregnancy continued uneventfully and resulted in a normal vaginal delivery of a healthy newborn. Before her discharge from the hospital after the birth, our patient started having severe pelvic pain. Imaging showed multiple possible bone tumors, and after the patient underwent a biopsy, she received a diagnosis of a metastatic neuroendocrine carcinoma of unknown origin. We then recognized that the multiple cytogenetic abnormalities in circulating tumor DNA caused the abnormal cfDNA result. Our patient, a 37-year-old mother of two, has since died from the cancer.

Since 2013, more cases of maternal cancer that were incidentally identified with the use of cfDNA have been reported, usually after nonreportable (i.e., the fetal aneuploidy status could not be assessed) results have been obtained or when multiple aneuploidies have been detected that were inconsistent with a viable fetus.[2] The expected incidence of cancer diagnosis during pregnancy is 1 in 1000 persons, but because cfDNA screening is now the standard for aneuploidy screening and is offered to millions of women worldwide, more cases of maternal cancer are being identified. Evidence-based guidelines regarding a standardized patient evaluation in the context of multiple aneuploidies on cfDNA screening are lacking,[3] and educating obstetrical providers about the possibility of incidentally identifying a maternal cancer through cfDNA screening has been challenging.[4]

In this issue of the *Journal*, Turriff et al.[5] report on the sensitivity of nonreportable or unusual cfDNA screening results in the actual detection of cancer in pregnant patients. Their study also provides guidance on the evaluation of pregnant persons with a nonreportable result or unusual results. They defined cfDNA screening results suggestive of maternal cancer as those that were abnormal and inconsistent with a viable fetus on ultrasonography, abnormal and discordant with the fetal karyotype or chromosome microarray analysis, or nonreportable.

In this prospective cohort study involving pregnant persons with cfDNA results suggestive of cancer, almost half the participants in the initial cohort (52 of 107 participants; 48.6%) received a diagnosis of cancer, with lymphoma being the most common diagnosis (31 of the 52 participants;