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Review

Chronic Viral Infections and Hepatic Oncogenesis

Radostina Alexandrova[1*], Hristo Hristov[1], Plamen Kirov[1], Abedulkadir Abudalleh[1], Tanya Zhivkova[1], Lora Dyakova[2], George Dimitrov[3]

[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3]*Department of Medical Oncology, Medical University of Sofia, Tsaritsa Yoanna Hospital, Sofia, Bulgaria*

Abstract

Hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatitis D virus (HDV) are major contributors to chronic liver disease and hepatocellular carcinoma (HCC) worldwide. Discovered in the 1960s and 1980s, these viruses are associated with significant global health burdens. HBV, a member of the Hepadnaviridae family, is a partially double-stranded DNA virus responsible for chronic liver infections that can lead to HCC. Despite the success of HBV vaccination, challenges such as vaccine escape mutants and non-responders persist. HCV, a member of the Flaviviridae family and a positive-sense single-stranded RNA virus, affects millions globally. While direct-acting antivirals (DAAs) can achieve high rates of viral clearance, vaccine development remains hindered by genetic diversity and immune evasion. HDV, the smallest known human virus, relies on HBV for its propagation. It exacerbates liver disease and increases HCC risk, particularly in co-infected individuals. HDV does not integrate into the host genome, suggesting indirect mechanisms of carcinogenesis, potentially through interactions with HBV. Addressing these challenges requires enhanced vaccination strategies, improved antiviral therapies, and continued research to understand the complex interplay between these viruses and liver cancer development.

Keywords: virus-induced oncogenesis, hepatocellular carcinoma, hepatitis viruses, antiviral treatment, vaccines

Резюме

Хепатит В вирусът (HBV), Хепатит С вирусът (HCV) и хепатит D вирусът (HDV) са сред основните фактори допринасящи за развитието на хронично чернодробно заболяване и хепатоцелуларен карцином (HCC) в световен мащаб. Открити през 60-те и 80-те години на миналия век, тези вируси са свързани със значителен глобален здравен проблем. HBV, член на семейство Hepadnaviridae, чийто геном е частично двуверижна ДНК молекула, е отговорен за хронични чернодробни инфекции, които могат да доведат до HCC. Въпреки успеха на ваксинацията срещу HBV, тя продължава да е изправена пред предизвикателства като „избягали от ваксината мутанти“ и т.нар. нон-респондъри, т.е. хора, които не са успели да изградят оптимален имунен отговор след ваксинацията. HCV, принадлежащ на семейство Flaviviridae вирус с геном, представен от положителна едноверижна РНК молекула, засяга милиони по света. Докато антивирусните лекарства с директно действие (DAA) могат да постигнат високи нива на изчистване на вируса, създаването на ваксина остава възпрепятствано от генетичното разнообразие на вируса и способността му да бяга от имунния отговор. HDV, най-малкият известен човешки вирус, разчита на HBV за своето разпространение. Той обостря чернодробното заболяване и повишава риска от HCC, особено при коинфектирани с HBV лица. HDV не се интегрира в генома на гостоприемника, което предполага индиректни механизми на канцерогенеза, потенциално чрез взаимодействия с HBV. Справянето с тези предизвикателства изисква подобрени стратегии за ваксиниране, усъвършенствани антивирусни терапии и продължаващи изследвания, за да се разбере сложното взаимодействие между тези вируси и развитието на рак на черния дроб.

*Corresponding author; rialexandrova@hotmail.com
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Infection-attributable malignancies

Infectious agents, including viruses, bacteria, and parasites, are responsible for up to more than 20% of the global cancer burden (zur Hausen, 2009; Plummer *et al.,* 2016; Schiller and Lowy, 2021). In 2018, approximately 2.2 million cancer cases attributable to infections were diagnosed worldwide. These cases were primarily associated with Helicobacter pylori (810,000 cases), human papillomavirus (HPV, 690,000 cases), hepatitis B virus (HBV, 360,000 cases), and hepatitis C virus (HCV, 160,000 cases) (de Martel *et al*., 2020).

The highest burden of infection-attributable cancers is observed in Eastern Asia and sub-Saharan Africa, whereas Northern Europe experiences the lowest burden (de Martel *et al*., 2020). Around 85% of virus-induced cancers are diagnosed in developing regions. Certain viruses exhibit gender-specific oncogenic potential; nearly 90% of cancers caused by HPV occur in women, while approximately two-thirds of HBV, HCV, and Epstein–Barr virus (EBV)-associated cancers are found in men (Schiller and Lowy, 2021).

The primary human oncogenic viruses include HPV, HBV, HCV, EBV, Kaposi's sarcoma-associated herpesvirus (KSHV or human herpesvirus 8), human T-cell lymphotropic virus (HTLV-1), and Merkel cell polyomavirus (MCPyV) (Schiller *et al*., 2021; Galati *et al*., 2024). All except MCPyV are classified by the International Agency for Research on Cancer (IARC) as Group 1 carcinogens, meaning they are known to be carcinogenic to humans. MCPyV is classified as Group 2A, indicating it is probably carcinogenic to humans, while BK polyomavirus (BKPyV) and John Cunningham polyomavirus (JCPyV) are classified as Group 2B, or possibly carcinogenic to humans. The role of these polyomaviruses in human carcinogenesis remains less well-defined, as cancers associated with them predominantly occur in immunocompromised individuals (Lee and Langhoff, 2006; Prado *et al*., 2018; Lewis *et al*., 2023; Galati *et al*., 2024).

Although human immunodeficiency virus (HIV) is not directly oncogenic, it significantly increases susceptibility to developing a malignancy caused by oncogenic viruses. Consequently, HIV-1 is classified as a Group 1 carcinogen (Anampa *et al*., 2020; Omar *et al*., 2024; Galati *et al*., 2024). The incidence of Kaposi's sarcoma surged following the HIV pandemic but decreased markedly with the advent of combination antiretroviral therapy (Grabar and Costagliola, 2021). HIV-2 is classified as Group 2B by the IARC (IARC, 2024).

Human oncogenic viruses are diverse, originating from six families with varying genomes, morphologies, replication strategies, transmission routes, cellular tropisms, cancer pathologies, and prevalence rates (Table 1). Despite these differences, they share common characteristics: they are specific to humans, establish chronic infections that persist for years without obvious symptoms, and evade the host immune response, which prevents the clearance of the infection. Immunosuppression is recognized as a factor that favors carcinogenesis (Mesri *et al*., 2014; Krump and You, 2018; Galati *et al*., 2024).

Cancers often develop in chronically infected individuals many years after the initial infection. For instance, hepatotropic oncoviruses like HBV and HCV typically lead to hepatocellular carcinoma 20-30 years after the initial infection (Ng and Wu, 2012). In contrast, Kaposi's sarcoma, induced by human herpesvirus 8 (HHV-8), can develop within months of infection, particularly in immunocompromised individuals (Cesarman *et al*., 2019).

Some of these viruses, such as EBV and HPV, are widespread in the general population, but the incidence of associated cancers is relatively low (Galati *et al*., 2024). Cancer development is observed in only a small fraction of infected individuals, as oncogenic viruses are necessary but not sufficient for cancer development (Mesri *et al*., 2014). Carcinogenesis does not benefit the virus; rather, it may be detrimental. For example, the integration of HPV into the host genome in cervical carcinoma can result in the loss of some viral genetic material, leading to a non-productive infection and an incomplete viral life cycle (Krump and You, 2018; Galati *et al*., 2024).

Hepatic malignancies

Liver cancer is a major global health issue, with 905,700 new cases and 830,200 deaths reported in 2020. It ranks among the top three causes of cancer-related mortality in 46 countries and among the top five in 90 countries (Rumgay *et al*., 2020; Llovet *et al*., 2021).

Hepatocellular carcinoma (HCC) is the predominant form of liver cancer in adults, representing approximately 90% of cases (Llovet *et al*., 2021; Yin and Kan, 2023; Kocarnik *et al*., 2024). In children, the most prevalent type of liver cancer is hepatoblastoma (Kocarnik *et al*., 2024).

The prognosis for liver cancer remains poor, with a 5-year survival rate of only 18% (Villanueva, 2019). The high mortality rate is largely attributed to the lack of sensitive biomarkers for early detec-

Table 1. Oncogenic human viruses

Virus	Family	Genome (size)	Virion structure	Tropism	Cancers induced	Most Affected Regions
High-risk human papillomaviruses (HPV)	Papillomaviridae	dsDNA (8.0 kb)	Non-enveloped viruses with an icosahedral capsid	Keratinocytes	Cervical, anal, vulva, vagina, penis, and head and neck cancers	Central America, South America, Sub-Saharan Africa, Regions of Asia
Merkel cell polyomavirus (MCPyV)	Polyomaviridae	dsDNA (5.4 kb)	Non-enveloped viruses, icosahedral capsid	Epidermal keratinocytes and dermal fibroblasts	Merkel cell carcinoma	North America, Australia, Europe
Epstein–Barr virus (EBV/HHV-4)	Herpesviridae	dsDNA (172 kb)	Enveloped viruses, icosahedral capsid	Epithelial cells and B cells	Hodgkin lymphoma, Burkitt lymphoma, nasopharyngeal carcinoma, 10% of gastric carcinoma	East Asia, East Africa, Regions of the Americas
Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8)	Herpesviridae	dsDNA (140 kb)	Enveloped viruses, icosahedral capsid	Epithelial cells and B cells	Kaposi sarcoma	Regions of Europe, Sub-Saharan Africa
Hepatitis B virus (HBV)	Hepadnaviridae	Partially dsDNA (3.3 kb)	Enveloped viruses, icosahedral capsid	Hepatocytes	Hepatocellular carcinoma; associated with other non-hepatocellular malignancies	Asia, Sub-Saharan Africa, South America
Hepatitis C virus (HCV)	Flaviviridae	Positive-sense ssRNA (9.6 kb)	Enveloped viruses, cosahedral capsid	Hepatocytes and extrahepatic cells	Hepatocellular carcinoma; associated with extrahepatic cancers such as non-Hodgkin lymphoma	Regions of Asia, the Americas, North Africa, Mediterranean
Human T-cell lymphotropic virus (HTLV-1)	Retroviridae	Two covalently bound positive-sense RNA strands (9.0 kb)	Enveloped viruses, icosahedral capsid	T and B cells	Adult T-cell leukemia and lymphoma	Japan, Australia, Regions of Africa, South America, Middle East

According to Krump, You, 2018; Schiller and Lowy, 2021; Zella and Gallo, 2021; Ameya and Birri, 2023; Galati *et al*., 2024; ds – double stranded; ss-single stranded.

tion, the absence of effective treatment strategies for advanced HCC, and the frequent occurrence of relapse [Wang and Wei, 2020]. The median survival for patients with early-stage HCC exceeds 60 months but drops to less than 15 months when diagnosed at an advanced stage (Parikh *et al*., 2020).

More than 90% of HCC cases arise in the context of chronic liver disease, which can be caused by various factors. The principal risk factors include hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatitis D virus (HDV) infections, as well as alcohol abuse, nonalcoholic fatty liver disease, microcystins (toxins produced by cyanobacteria), long-term consumption of aflatoxin-contaminated food, and smoking. Conditions such as primary biliary cholangitis, hemochromatosis, and

Table 2. Hepatitis human viruses associated with liver malignancies

Name	Hepatitis B virus	Hepatitis C virus	Hepatitis D virus	Hepatitis E virus
Discovery	1963	1989	1977	1983
Classification	Hepadnaviridae	Flaviviridae	Kolmioviridae	Hepeviridae
Genotypes	Ten genotypes (A – J)	Seven major genotypes (1-7)	Eight major genotypes (1 – 8)	Eight genotypes, four of them infect humans (1 – 4)
Genome	Circular, partially ds DNA genome (~3.2 kb)	/+/sense ssRNA (~9.6 kb)	Circular /-/sense RNA (1.7 kb)	/+/sense ssRNA (~7.5 kb)
Transmission routes	Primarily involving exposure to infectious blood or body fluids	Parenteral routes	Parenteral routes	Primarily waterborne and zoonotic, with additional routes including foodborne, parenteral, and vertical transmission
Incubation period (days)	60 - 150	14 - 180	21 - 49	14 - 63
Ability to induce chronic infection	Yes	Yes	Yes	In immunocompromised people
Oncogenic potential	Yes	Yes	Yes	Controversial
Oncoproteins	HBx	Core, NS3, Ns5A	-	-
Targets in the cell	p53, pRB, Wnt, Src, DNMTs, Ras, PI3K, JNK, NF-κB, ERK, TGFβ, HDACs	p53, PARP, hTERT, TGFβ, HDACs	SHH, GADD45	-
Availability of vaccine	Yes	No	No	Yes (licensed in China)
Treatment	-Interferons (eg. Interferon-α and Pegylated Interferon-α) and -Nucleos(t)ide analogues (eg. Lamivudine, Entecavir)	Three generations of DAAs (eg. Glecaprevir / pibrentasvir)	PEG-IFNα, Bulevirtide, Lonafarnib.	No specific antiviral treatment Ribavirin PEG-IFNα
References	Galati *et al*., 2024; Mironova and Ghany, 2024; Gerlich, 2013	Houghton Galati *et al*., 2024	Ferenci *et al*., 2022 Negro and Lok, 2023	Abravanel *et al*., 2023; Alexandrova *et al*., 2024

ss – single stranded; ds – double stranded; DAAs = direct-acting antivirals

α1-antitrypsin deficiency also contribute to an increased risk of cirrhosis and HCC (Llovet *et al*., 2021; Yin and Kan, 2023; Kocarnik *et al*., 2024).

Patients with cirrhosis due to hereditary hemochromatosis are particularly susceptible to HCC, with up to 45% developing the disease during their lifetime due to genetic predisposition and iron overload (Fracanzani *et al*., 2001; Llovet *et al*., 2021; Haider *et al*., 2022).

With approximately 2 billion adults classified as obese or overweight and over 400 million having diabetes (Asrani *et al*., 2019), obesity-related conditions such as non-alcoholic steatohepatitis and type 2 diabetes are emerging as rapidly growing etiologies of HCC, especially in Western countries (Talamantes *et al*., 2023).

Notably, around 80% of HCC cases are attributed to HBV and HCV infections. Hepatitis D virus (HDV) infection also significantly contributes to HCC progression, particularly in patients co-infected with HBV (Plummer *et al*., 2016; Yin and Kan, 2023; Lombardo *et al*., 2024). Additionally, there

is emerging evidence suggesting a potential role of hepatitis E virus (HEV) in hepatocarcinogenesis (Yin and Kan, 2023; Alexandrova *et al*., 2024). Comparative data about these hepatitis viruses are presented in Table 2. Various factors including age, ethnicity, gender, lifestyle, regional distribution, and the stage of underlying liver disease influence the development of HCC (Omar *et al*., 2023). The incidence of HCC is rare before the age of 40 but increases with age, peaking around 70 (Omar *et al*., 2023).

HCC incidence and mortality rates are two to four times higher in males compared to females across all regions, with the disparity being more pronounced in high-risk areas (Rumgay *et al*., 2020; Yin and Kan, 2023; Omar *et al*., 2023). This disparity may be partially explained by greater male exposure to risk factors such as alcohol and smoking, as well as the protective effects of estrogen against HCC through mechanisms such as IL-6 restriction, STAT3 inactivation, and tumor-associated macrophage inhibition. In contrast, androgen/AR signaling is implicated in the initiation of HCC related to carcinogens or hepatitis B virus (Shi *et al*., 2014; Ma *et al*., 2014; Harding and Heaton, 2022; Omar *et al*., 2023).

Approximately 80% of HCC cases occur in low- and middle-income countries, particularly in sub-Saharan Africa and Eastern Asia, regions with high prevalences of chronic HBV infection. Incidence and mortality rates are also rising in parts of Europe and the USA (Llovet *et al*., 2021; Yin and Kan, 2023; Kim, 2024; Abboud *et al*., 2024).

Projections suggest that unless we turn the tide, viral hepatitis will kill more people worldwide by 2040 than human immunodeficiency virus (HIV) infection, tuberculosis, and malaria combined (Foreman *et al*. 2018; Cox *et al*., 2020). This highlights the need to better understand the biology and pathogenicity, including the oncogenic potential, of these viruses and to improve diagnostic, prophylactic, and therapeutic approaches and their application.

Hepatitis B virus

The hepatitis B virus (HBV) was discovered in 1963 by Dr. Baruch Blumberg, who was awarded the Nobel Prize in Physiology or Medicine in 1976 for this achievement (Blumberg, 2002; Gerlich, 2013). HBV is a member of the Hepadnaviridae family and primarily targets hepatocytes in the liver. The complete virion, known as the Dane particle, is spherical, has an envelope, and measures approximately 42 nm in diameter. Additionally, electron microscopy reveals two smaller, non-infectious particles: spherical particles with a diameter of 20 nm and filamentous particles with a width of 22 nm. These particles consist of hepatitis B surface antigen (HBsAg) complexed with host lipids but lack viral DNA (Li *et al*., 2021; Liu *et al*., 2021; Bello *et al*., 2023; Toyé *et al*., 2023; Mironova and Ghany, 2024).

The HBV genome is composed of partially double-stranded DNA (3.2 kb) and includes four overlapping open reading frames (ORFs): C, S, P, and X, which encode for core/HBeAg, surface, polymerase, and X proteins, respectively. To date, HBV has been classified into 10 genotypes (A–J), with subgenotypes further subdividing most of these genotypes (except E, G, and J) (Liu *et al*., 2021). At least 30 subgenotypes have been identified, showing 4–7.5% divergence and varying in ethno-geographical distribution and clinical outcomes. HBV also has four serotypes: adw, ayw, ayr, and adr, which share a common determinant 'a' recognized by neutralizing antibodies (anti-HBs), thus providing broad protection through the HBV vaccine (Zhang *et al*., 2016; Li *et al*., 2021; Liu *et al*., 2021; Bello *et al*., 2023; Toyé *et al*., 2023; Mironova and Ghany, 2024).

The HBV reverse transcriptase lacks proofreading activity, leading to a high mutation rate, estimated at 1.4 to 3.2 × 10^−5 substitutions per site per year (Liu *et al*., 2021). Globally, more than 2 billion people are estimated to be affected by HBV, with up to 300 million suffering from chronic liver disease due to HBV infection (Agustiningsih *et al*., 2024). Chronic HBV infection carries a 10–25% lifetime risk of developing hepatocellular carcinoma (HCC) (Flores *et al*., 2022; Mironova and Ghany, 2024).

The likelihood of chronic HBV infection is inversely related to the age at which the infection occurs. Up to 90% of infants infected with HBV within the first 12 months of life develop chronic infection, compared to 25–50% of children infected before age 5, and 5–10% of infected adults (Komatsu *et al*., 2017; Flores *et al*., 2022; Mironova and Ghany, 2024). A large epidemiological study in China found that HBV-related HCC often presents approximately 10 years earlier than HCC associated with other risk factors, such as HCV, alcohol abuse, diabetes, or aflatoxin B1 consumption. Additionally, HBV-related HCC is characterized by higher α-fetoprotein levels and more pronounced microvascularity (Yang *et al*., 2019).

Several mechanisms contribute to HBV's

role in HCC pathogenesis:

• Integration of HBV DNA: During viral replication, HBV DNA integrates into the host genome, causing genomic instability and insertional mutagenesis, which impairs the expression of proto-oncogenes and tumor suppressor genes.

• Chronic Inflammation: Chronic HBV infection induces proinflammatory cytokine production and oxidative stress, creating an inflammatory microenvironment that damages the liver. This inflammation, combined with liver injury and regeneration, is a key factor in HCC development.

• HBV X Protein (HBx): HBx interacts with various cellular factors, including non-coding RNAs, and disrupts the expression of cellular genes, leading to malignant transformation. It affects processes such as transcription, signal transduction, cell cycle regulation, apoptosis, autophagy, protein degradation, and genetic stability (Komatsu *et al*., 2017; Li *et al*., 2021; Agustiningsih *et al*., 2024).

Chronic HBV infection has been associated with several malignant disorders including lymphoma, and biliary, cervical, uterine, breast, thyroid, lung, and skin cancers (An *et al*., 2018).

The HBV vaccine has been in use worldwide for over four decades. The first vaccine, developed in France and the USA in the early 1980s, was a plasma-derived vaccine based on HBsAg extracted from the plasma of asymptomatic carriers. Plasma-derived vaccine demonstrated excellent safety and efficacy but was replaced by a second-generation recombinant vaccine in 1986, produced using genetic engineering techniques in yeast. This vaccine represents the first of its kind globally. In 2018, a further advancement was made with the introduction of a vaccine that includes an adjuvant, enabling a two-dose regimen instead of the previous three-dose schedule (Szmuness *et al*., 1980; McAleer *et al*., 1984; Francis *et al*., 1986; Romano and Zanetti, 2022; Al-Busafi and Alwassief, 2024).

Currently, HBV vaccines are available in single- or triple-antigen forms and in combination with vaccines against other infections. Over one billion doses have been administered since 1982, demonstrating an excellent safety profile. Adverse effects are typically mild and transient, such as injection site reactions. The vaccine is also deemed safe for pregnant and lactating women, low birth weight infants, and individuals with HIV (Al-Busafi and Alwassief, 2024; Mironova and Ghany, 2024). Although the duration of protection is not precisely defined, it is estimated to last at least 30 years (Al-Busafi and Alwassief, 2024).

By the end of 2020, 190 WHO Member States had adopted universal hepatitis B vaccination for infants, with an estimated global coverage of 83% for the three-dose regimen. In 113 of these countries, the first dose is administered within the first 24 hours of birth (Flores *et al*., 2022). This has led to a sustained reduction in mother-to-child transmission, chronic hepatitis B, and HCC, making the HBV vaccine the first anticancer vaccine (Mironova and Ghany, 2024). Despite this, global birth dose coverage remains suboptimal, with the WHO setting a target of 90% vaccination coverage to achieve hepatitis B elimination by 2030 (Flores *et al*., 2022; Mironova and Ghany, 2024).

Current scientific evidence suggests that routine booster doses of the HBV vaccine are not necessary for fully vaccinated, healthy, immunocompetent individuals. However, booster doses may be recommended for specific groups, such as immunocompromised individuals or healthcare workers at higher risk of HBV exposure (Al-Busafi and Alwassief, 2024).

Challenges to vaccine efficacy include the emergence of vaccine escape mutants (Al-Busafi and Alwassief, 2024) and vaccine non-responders—individuals who develop an anti-HBs titer below 10 mIU/mL after complete vaccination. Approximately 10% of vaccine recipients worldwide fall into this category. Risk factors for non-response include older age at immunization, obesity, smoking, comorbidities (such as diabetes, chronic kidney disease, and chronic liver disease), and immune suppression. Timely detection and strategies to optimize immune response, such as additional booster doses, are needed for these individuals (Al-Busafi and Alwassief, 2024).

Currently, two interferon-based drugs and seven oral nucleoside/nucleotide analogs (NAs) are approved for the treatment of chronic hepatitis B (Chien and Liaw, 2022).

Hepatitis C virus

Hepatitis C virus (HCV) was discovered by Choo and colleagues in 1989 as the primary cause of transfusion-associated non-A, non-B hepatitis (Choo *et al*., 1989). HCV is a member of the Flaviviridae family, characterized as an enveloped, positive-sense, single-stranded RNA virus with a diameter of approximately 60 nm. Its genome is about 9.6 kb long and encodes a single polyprotein, which is subsequently cleaved by viral and cellular proteases to produce structural proteins (core and envelope glycoproteins E1 and E2) and non-structural (NS) proteins (Choo *et al*., 1989; Tsukiyama-Koha-

ra and Kohara, 2018; Manne *et al*., 2021).

Approximately 25% of patients with acute HCV infection experience spontaneous viral clearance. In contrast, chronic hepatitis C usually progresses slowly over the first two decades but can accelerate due to factors such as advancing age, heavy alcohol consumption, and co-infection with HIV. Among chronically infected individuals, at least 30% will develop liver fibrosis, 7–18% will progress to cirrhosis, and 1–5% will develop hepatocellular carcinoma within 20–30 years (Hajarizadeh *et al*., 2013; Reungoat *et al*., 2021). Despite the availability of highly effective direct-acting antivirals (DAAs) that achieve sustained virological response (SVR) in over 95% of treated patients, an estimated 57 million people were living with HCV infection globally in 2020. In 2021, approximately 150,000 deaths due to HCV-associated HCC were reported, the highest number on record (Fiehn *et al*., 2024).

Epidemiological studies and meta-analyses suggest an increased rate of extrahepatic cancers in patients with chronic HCV infection along with a higher risk of intrahepatic cholangiocarcinoma, pancreatic cancer and non-Hodgkin lymphoma (Pol *et al*., 2018). HCV does not integrate into the host genome and cannot stably persist as an episome. However, several HCV proteins (including core and NS proteins 3 and 5A) influence host signaling pathways that promote carcinogenesis by enhancing cell proliferation and survival. HCV targets include epidermal growth factor (EGF), signal transducer and activator of transcription 3 (STAT3), transforming growth factor beta (TGF-β), and vascular endothelial growth factor (VEGF). By manipulating these pathways, HCV promotes its replication and persistence, which has significant implications for viral pathogenesis and oncogenesis. Additionally, HCV induces epigenetic dysregulation and contributes to immune-mediated inflammation (Goto *et al*., 2020; Fiehn *et al*., 2024).

One of the most important questions related to the use of DAAs in the treatment of chronic hepatitis C is their impact on hepatocellular carcinoma occurrence or recurrence and in particular whether the treatment can completely restore the balance in the liver (in which cases, to what extent) and eliminate the risk of cancer development and progression. The answer still remains unclear due to various challenges including the presence of non-viral factors contributing to liver deterioration and the varying response of patients related to regression of fibrosis in response to therapy as well as the long period required for cancers to appear (Waziry *et al*., 2017; Goto *et al*., 2020; Reungoat *et al*., 2021, Sapena *et al*., 2022; Celsa *et al*., 2022).

Developing a vaccine for hepatitis C has proven challenging due to several factors:

• Genetic Heterogeneity: HCV is highly variable, with six major genotypes (differing by ~25–35% at the nucleotide level) and over 100 subtypes (differing by ~15–25%). The polyprotein sequences of diverse isolates differ by about 30%, and envelope proteins (E1 and E2) differ by up to 50%. This genetic diversity, exacerbated by an error-prone polymerase that generates viral variants, means that HCV circulates as a quasispecies—a complex mixture of closely related but distinct genomes (Tsukiyama-Kohara and Kohara, 2017; Echeverría *et al*., 2021] The Lancet Gastroenterology Hepatology, 2021; Malaina *et al*., 2023). Developing a global vaccine requires protection against all these variants.

• Immune Evasion: HCV has evolved mechanisms to evade the host immune response, complicating vaccine development (Alizei *et al*., 2021).

• Experimental Models: Research is hampered by the lack of suitable in vitro and in vivo models. Although hepatitis C infection in chimpanzees closely mirrors that in humans, ethical concerns and high costs limit the use of these animals for research (Berggren *et al*., 2020).

• Clinical Trials: Conducting clinical trials for HCV vaccines is challenging due to the relatively low incidence of HCV in many industrialized countries and the difficulty in selecting high-risk populations for vaccine testing. High-risk groups include those requiring blood transfusions, injecting drug users, and healthcare workers with frequent exposure to blood (The Lancet Gastroenterology Hepatology, 2021; Malaina *et al*., 2023).

The only phase II clinical trial of an HCV vaccine candidate to date targeted individuals who inject drugs to prevent chronic infection in this high-risk group. While the vaccine elicited a specific T-cell immune response and reduced viral RNA levels, it unfortunately failed to prevent chronic HCV infection (Hartlage and Kapoor, 2021).

Despite successful antiviral therapies, the development of an HCV vaccine remains a priority. This is due to factors such as the large number of people who are unaware of their HCV infection, the high cost of treatment limiting access, advanced liver damage in some chronic HCV cases that may not reverse even after viral clearance, and the ongoing risk of re-infection among high-risk populations (Cox, 2020; Malaina *et al*., 2023).

Hepatitis D virus

Hepatitis D virus (HDV) was discovered in 1977 by Rizzetto and colleagues in patients with chronic hepatitis B virus infection. It was initially thought to be an unknown antigen of the HBV virus (Rizzetto *et al*., 1977). HDV is the smallest known human virus and is a satellite virus of the hepatitis B virus, relying on envelope proteins of the HBV to maintain its productive infection.

HDV belongs to the family Kolmioviridae and its genome is a negative-sense circular RNA molecule (1.7-kb) complexed with the sole viral-encoded protein, the delta antigen (HDAg). The delta antigen is present in two forms: the small HDAg (S-HDAg), essential for viral replication, and the large HDAg (L-HDAg), important for virion assembly. The diameter of the virion is 35–37 nm. HDV is considered a hybrid virus as it uses HBV surface antigen as its envelope protein (Smedile and Verme,1999; Rizzetto *et al*., 2015; Puigvehí *et al*., 2019; Lombardo *et al*., 2024; Juang *et al*., 2024). HDV infection can manifest as co-infection (simultaneous HBV and HDV infection) or superinfection (HDV infects someone already chronically infected with the hepatitis B virus) (Juang *et al*., 2024).

Eight distinct HDV genotypes have been recognized, with genetic sequence similarities ranging between 81% and 89% (Le Gal *et al*., 2017; Lombardo *et al*., 2024; Juang *et al*., 2024). Information on the number of people infected with the hepatitis D virus worldwide is quite heterogeneous, which is probably due to differences in the methodology used and a lack of data. According to three meta-analyses, the total number of such patients in three consecutive years was as follows: between 48–60 million in 2018, 62–72 million in 2018, and 12 million people in 2019 (Chen *et al*., 2019; Miao *et al*., 2020; Stockdale *et al*., 2020). According to the data of the World Health Organization (WHO), there are at least 20 million people infected with HDV worldwide, which represents 5% of HBV carriers (World Health Organization, 2017).

Hepatitis D occurs throughout the globe, but control of the hepatitis B virus in the last decades has consistently diminished the circulation of HDV in industrialized countries. However, hepatitis D continues to be a medical problem for some at-risk groups, such as injecting drug users. Unlike HBV, vertical mother-to-offspring transmission, homosexual promiscuity, or nosocomial exposure appear to be very underrepresented risk factors for HDV transmission (Rizzetto *et al*., 2015).

Hepatitis D virus infection increases the risk of developing cirrhosis, liver failure, and hepatocellular carcinoma in the natural course of chronic hepatitis B patients compared with HBV mono-infection. Furthermore, HDV-associated HCC often appears at a younger age and exhibits more aggressive behavior (Tamura *et al*., 1993; Rizzetto and Alavian 2013; Alfaiate *et al*., 2020; Lombardo *et al*., 2024). In Greece, Samoa, and the Far East, HDV is associated with benign clinical conditions or normal liver function, suggesting that disease presentation may vary, possibly due to different HDV genotypes (Rizzetto and Ciancio 2012). It has been reported that HDV viremia increases the risk of HCC and plays an essential role in liver cancer development in chronic hepatitis B patients treated with NAs (Kamal *et al*., 2020; Jang *et al*., 2021). L-HDAg has been suggested to influence host signaling pathways, potentially contributing to HDV pathogenesis. For example, the expression of proteins responsible for epithelial-to-mesenchymal transition is upregulated by L-HDAg, which may be involved in progressive fibrosis (Goodrum and Pelchat, 2018; Liang *et al*., 2020; Juang *et al*., 2024).

HBV/HDV-coinfected patients seem to be at an increased risk of HCC development compared to HBV-monoinfected individuals, although the evidence is still limited and the molecular alterations present in patients with HDV-related HCC are not clarified. Thus, HDV is not yet included on the list of oncogenic agents, whilst HBV and HCV are well-defined carcinogens (IACR, 2024; Puigvehí *et al*., 2019). There are very limited data on the potential oncogenic mechanisms of HDV.

HDV does not integrate into the genome and lacks the machinery required to propagate in the absence of HBV. Therefore, a direct oncogenic mechanism of HDV is unlikely. It is possible, however, that interactions between HDV and HBV promote the development of HCC.

Various potential mechanisms by which HDV can modify key signaling pathways related to fibrosis and cancerogenesis, including epigenetic changes and immune response modifications have been proposed. Among them is enhanced transforming growth factor-β (TGF-β) signaling. TGF-β is involved in multiple cellular processes, such as growth, differentiation, wound repair, and apoptosis, with a regulatory role in fibrosis and hepatocarcinogenesis. The ability of HDV to interfere with DNA methylation also requires attention because the silencing of tumor-suppressor genes through this modification is a frequent epigenetic event

in HCC (Puigvehí *et al.,* 2019; Farci *et al*., 2021; Juang *et al*., 2024).

To date, we do not have a therapeutic strategy that completely cures chronic hepatitis D. Treatment with interferon or pegylated interferon alfa achieves the sustained suppression of HDV replication in only 25% of patients. Nucleos(t)ide analogs used in the treatment of HBV have no antiviral activity against HDV. This highlights the need for effective new therapies for HDV (Ferenci *et al*., 2022).

Hepatitis E virus

Hepatitis E was first recognized by Mohammed Khuroo during an epidemic of hepatitis in Kashmir Valley, India, in 1978. Hepatitis E virus (HEV) was discovered in 1983 by Mikhail Balayan, who was investigating an outbreak of unexplained hepatitis in Russian soldiers stationed in Afghanistan. During this outbreak, a pooled fecal extract from affected soldiers was ingested by a member of the research team, who subsequently developed hepatitis. The virus, later named HEV, was detected in his stool by electron microscopy. This discovery was pivotal in identifying HEV as the causative agent of what was previously known as enterically transmitted non-A, non-B hepatitis (Balayan *et al*., 1983; Khuroo, 2016; Kamar *et al*., 2017).

The Hepatitis E virus belongs to the Hepeviridae family and is a small virus (32-34 nm) with a spherical shape, icosahedral symmetry, and a single-stranded, positive-sense RNA genome. HEV is a quasi-enveloped virus because it is non-enveloped in feces and bile but contains a lipid envelope in blood and in infected cell cultures. Non-enveloped HEV particles are stable in the environment, and through them, HEV infects other hosts. Enveloped virions do not contain virus-encoded proteins and this helps them to avoid the attack of neutralizing antibodies in the host, to enter new cells, and to start new replication cycles (Alexandrova *et al*., 2024).

HEV is a major cause of acute viral hepatitis globally, with an estimated 20 million infections annually (Blasco-Perrin *et al*., 2016).

HEV is categorized into eight genotypes, four of which (HEV-1 to HEV-4) infect humans. HEV-7 infects dromedary camels, but very rare cases in humans have been reported as well. Genotypes 1 and 2 are human-specific and prevalent in developing countries, causing waterborne outbreaks. Genotypes 3 and 4 are zoonotic and affect both humans and animals, primarily in developed countries. Transmission occurs mainly via the fecal-oral route through contaminated water in endemic regions, while zoonotic transmission via undercooked meat and blood transfusions has been documented in developed areas (Kamar *et al*., 2017; Alexandrova *et al*., 2024).

The clinical spectrum of HEV infection ranges from asymptomatic to severe acute hepatitis (Aggarwal and Jameel, 2011). Although most infections are self-limiting, severe disease can be significant, particularly in pregnant women and those with preexisting liver conditions. Chronic HEV infection, particularly in immunocompromised individuals, poses a risk of cirrhosis. The potential link between HEV and HCC is emerging but remains controversial. Recent studies suggest a statistically significant association between HEV infection and increased HCC risk. This association is more pronounced when comparing HEV-infected individuals to healthy controls, but not significant when compared to patients with chronic liver diseases (Yin and Kan, 2023).

While these findings suggest a possible link between HEV and HCC, the precise mechanisms remain unclear. It is hypothesized that chronic HEV infection, particularly in immunocompromised individuals, may lead to persistent liver inflammation and subsequent carcinogenesis. The ability of HEV to disrupt the normal functioning of tumor suppressor genes and proto-oncogenes and interfere with key signaling pathways related to cell proliferation, apoptosis, oxidative stress, and angiogenesis has been reported (Klöhn, 2021). Further, well-designed studies are necessary to confirm these associations and elucidate the underlying mechanisms of HEV-related hepatocarcinogenesis.

Such investigations are challenging due to a number of factors, among which: chronic HEV infection occurs mainly in immunocompromised individuals and the total number of cases is low; globally, HEV infection is not routinely tested; difficulties related to experimental models (cell cultures, laboratory animals) suitable for cultivation of this virus.

Here are some questions that await answers: whether people in different parts of the world are affected to different degrees by the oncogenic potential of HEV; do HEV genotypes express different oncogenic potential? According to the available data, chronic HEV infection is primarily caused by HEV-3 and HEV-4 rather than HEV-1 and HEV-2; is there a relationship between HEV-induced neoplasia and some host characteristics (such as age, gender, lifestyle, comorbidities, etc.) (Klöhn, 2021; Alexandrova *et al*., 2024).

A recombinant three-dose vaccine against HEV genotype 1 was developed and is licensed in China in 2012 for immunizing adults 16 years old and above (Zhang *et al*., 2015).

The first-ever mass vaccination campaign against hepatitis E in response to an outbreak was implemented in 2022 in Bentiu internally displaced persons camp in South Sudan targeting 27,000 residents 16–40 years old, including pregnant women (Ciglenecki *et al*., 2022; Nesbitt *et al*., 2024).

Active work is also being done on the preparation of other vaccines against this virus (Hartley *et al*., 2024).

Conclusion

Hepatitis B, C, and D viruses pose significant global health challenges due to their roles in chronic liver disease and hepatocellular carcinoma (HCC). The hepatitis B virus (HBV) has been effectively managed through widespread vaccination, yet challenges like vaccine escape mutants and non-responders persist. Hepatitis C virus (HCV) continues to affect millions despite the success of direct-acting antivirals (DAAs), emphasizing the need for vaccine development and improved treatment access. Hepatitis D virus (HDV), which depends on HBV for replication, accelerates liver disease and increases HCC risk, particularly in co-infected individuals.

To mitigate the global burden of these viruses, ongoing efforts in prevention, vaccination, antiviral therapy, and novel research are crucial. Improving vaccine coverage, advancing treatment options, and understanding the complex interactions between these viruses will be key to reducing infection rates and preventing severe and irreversible liver complications.

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References

Abboud, Y., M. Ismail, H. Khan, E. Medina-Morales, S. Alsakarneh, F. Jaber, N. T. Pyrsopoulos (2024). Hepatocellular Carcinoma Incidence and Mortality in the USA by Sex, Age, and Race: A Nationwide analysis of two decades. *J. Clin. Transl. Hepatol.* **12**: 172-181. doi: 10.14218/JCTH.2023.00356.

Abravanel, F., S. Lhomme, O. Marion, J. M. Péron, N. Kamar, J. Izopet. (2023). Diagnostic and management strategies for chronic hepatitis E infection. *Expert. Rev. Anti Infect. Ther.* **21**: 143-148. doi:10.1080/14787210.2023.2166932.

Aggarwal, R, S. Jameel (2011). Hepatitis E. *Hepatology (Baltimore, Md.)*. **54**: 2218-2226. doi:10.1002/hep.24674.

Agustiningsih, A., M. R. Rasyak, S. Turyadi, C. Jayanti, C. Sukowati (2024). The oncogenic role of hepatitis B virus X gene in hepatocarcinogenesis: recent updates. *Explor. Target. Antitumor. Ther*. **5**: 120-134. doi: 10.37349/etat.2024.00209.

Al-Busafi, S. A., A. Alwassief (2024). Global perspectives on the hepatitis B vaccination: challenges, achievements, and the road to elimination by 2030. *Vaccines (Basel)* **12**: 288. doi: 10.3390/vaccines12030288.

Alexandrova, R., I. Tsachev, P. Kirov, A. Abudalleh, H. Hristov, T. Zhivkova, L. Dyakova, M. Baymakova (2024). Hepatitis E virus (HEV) infection among immunocompromised individuals: a brief narrative review. *Infect. Drug Resist.* **17**: 1021-1040. doi: 10.2147/IDR.S449221.

Alfaiate, D., S. Clément, D. Gomes, N. Goossens, F, Negro (2020). Chronic hepatitis D and hepatocellular carcinoma: A systematic review and meta-analysis of observational studies. *J. Hepatol.* **73**: 533-539. doi: 10.1016/j.jhep.2020.02.030.

Alizei, E. S., M. Hofmann, R. Thimme, C. Neumann-Haefelin (2021). Mutational escape from cellular immunity in viral hepatitis: variations on a theme. *Curr. Opin. Virol.* **50**: 110-118. doi: 10.1016/j.coviro.2021.08.002.

Ameya, G., D. J. Birri (2023). The molecular mechanisms of virus-induced human cancers. *Microb. Pathog*. **183**: 106292. doi: 10.1016/j.micpath.2023.106292.

An, J., J. W. Kim, J. H. Shim, S. Han, C. S. Yu, J. Choe, D. Lee, K. M. Kim, Y. S. Lim, Y. H. Chung, Y. S. Lee, D. J. Suh, J. H. Kim, H. C. Lee (2018). Chronic hepatitis B infection and non-hepatocellular cancers: A hospital registry-based, case-control study. *PLoS One*. **13**: e0193232. doi: 10.1371/journal.pone.0193232.

Anampa, J., S. K. Barta, M. Haigentz, J. A. Sparano (2020). 62 - Human Immunodeficiency Virus (HIV) Infection and Cancer. In: Niederhuber, J. E., J. O. Armitage, M. B. Kastan, J. H. Doroshow, J. E. Tepper (Eds). Abeloff's Clinical Oncology (Sixth Edition). Elsevier, London, pp. 894-903. https://doi.org/10.1016/B978-0-323-47674-4.00062-1.

Asrani, S. K., H. Devarbhavi, J. Eaton, P. S. Kamath (2019). Burden of liver diseases in the world. *J. Hepatol*. **70**: 151-171. doi: 10.1016/j.jhep.2018.09.014.

Balayan, M. S., A. G. Andjaparidze, S. S. Savinskaya, E. S. Ketiladze, D. M. Braginsky, A. P. Savinov, V. F. Poleschuk (1983). Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route. *Intervirol.* **20**: 23–31.

Bello, K. E., T. N. A. Mat Jusoh, A. A. Irekeola, N. Abu, N. A. Z. Mohd Amin, N. Mustaffa, R. H. Shueb (2023). A Recent prevalence of hepatitis B Virus (HBV) genotypes and subtypes in Asia: a systematic review and meta-Analysis. *Healthcare (Basel)* **11**: 1011. doi: 10.3390/healthcare11071011.

Berggren, K. A., S. Suzuki, A. Ploss (2020). Animal models used in kepatitis C virus research. *Int. J. Mol. Sci.* **21**: 3869. doi: 10.3390/ijms21113869.

Blasco-Perrin, H., F. Abravanel, V. Blasco-Baque, J. M. Péron (2016). Hepatitis E, the neglected One. *Liver Int.* **36**: 130-134. doi:10.1111/liv.13014.

Blumberg, B.S. (2002). Hepatitis B - The hunt for a killer virus. Princeton: Princeton University Press.

Celsa, C., C. Stornello, P. Giuffrida, C. M. Giacchetto, M. Grova, G. Rancatore, C. Pitrone, V. Di Marco, C. Cammà, G. Cabibbo (2022). Direct-acting antiviral agents and risk of hepatocellular carcinoma: Critical appraisal of the

evidence. *Ann. Hepatol.* **27**: 100568. doi: 10.1016/j.aohep.2021.100568.

Cesarman, E., B. Damania, S. E. Krown, J. Martin, M. Bower, D. Whitby (2019). Kaposi sarcoma. *Nat. Rev. Dis. Primers*. **5**: 9. doi: 10.1038/s41572-019-0060-9.

Chen, H. Y., D. T. Shen, D. Z. Ji, P. C. Han, W. M. Zhang, J. F. Ma, W. S. Chen, H. Goyal, S. Pan, H. G. Xu (2019). Prevalence and burden of hepatitis D virus infection in the global population: a systematic review and meta-analysis. *Gut* **68**: 512-521. doi: 10.1136/gutjnl-2018-316601.

Chien, R. N., Y. F. Liaw (2022). Current trend in antiviral therapy for chronic hepatitis B. *Viruses* **14**: 434. doi: 10.3390/v14020434.

Choo, Q. L., G. Kuo, A. J. Weiner, L. R. Overby, D. W. Bradley, M. Houghton (1989). Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* **244**: 359-62. doi: 10.1126/science.2523562.

Ciglenecki, I., J. Rumunu, J. F. Wamala, P. Nkemenang, J. Duncker, R. Nesbitt, E. Gignoux, T. Newport, M. Heile, C. Jamet, M. Rull, A.S. Azman (2022). The first reactive vaccination campaign against hepatitis E. *Lancet Infect. Dis*. **22**: 1110-1111. doi: 10.1016/S1473-3099(22)00421-2.

Cox, A. L. (2020). Challenges and promise of a hepatitis C virus vaccine. *Cold. Spring Harb. Perspect. Med*. **10**: a036947. doi: 10.1101/cshperspect.a036947.

de Martel, C., D. Georges, F. Bray, J. Ferlay, G. M. Clifford (2020). Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. *Lancet Glob. Health*. **8**: e180-e190. doi: 10.1016/S2214-109X(19)30488-7.

Echeverría, N., V. Comas, F. Aldunate, P. Perbolianachis, P. Moreno, J. Cristina (2021). In the era of rapid mRNA-based vaccines: Why is there no effective hepatitis C virus vaccine yet? *World J. Hepatol.* **13**: 1234-1268. doi: 10.4254/wjh.v13.i10.1234.

Farci, P., G. A. Niro, F. Zamboni, G. Diaz (2021). Hepatitis D virus and hepatocellular carcinoma. *Viruses* **13**: 830. doi: 10.3390/v13050830.

Ferenci, P., T. Reiberger, M. Jachs (2022). Treatment of chronic hepatitis D with bulevirtide-A fight against two foes-an update. *Cells* **11**: 3531. doi: 10.3390/cells11223531.

Fiehn, F., C. Beisel, M. Binder (2024). Hepatitis C virus and hepatocellular carcinoma: carcinogenesis in the era of direct-acting antivirals. *Curr. Opin. Virol.* **67**: 101423. doi: 10.1016/j.coviro.2024.101423.

Flores, J. E., A. J. Thompson, M. Ryan, J. Howell (2022). The global impact of hepatitis B vaccination on hepatocellular carcinoma. *Vaccines (Basel)* **10**: 793. doi: 10.3390/vaccines10050793.

Foreman, K. J., N. Marquez, A. Dolgert, K. Fukutaki, N. Fullman, M. McGaughey, M. A. Pletcher, A. E. Smith, K. Tang, C. W. Yuan et al. (2018). Forecasting life expectancy, years of life lost, and all-cause and cause-specific mortality for 250 causes of death: reference and alternative scenarios for 2016–40 for 195 countries and territories. *Lancet* **392**: 2052–2090. doi: 10.1016/S0140-6736(18)31694-5.

Fracanzani, A. L., D. Conte, M. Fraquelli, E. Taioli, M. Mattioli, A. Losco, S. Fargion (2001). Increased cancer risk in a cohort of 230 patients with hereditary hemochromatosis in comparison to matched control patients with non-iron-related chronic liver disease. *Hepatology* **33**: 647-651. doi: 10.1053/jhep.2001.22506.

Francis, D. P., P. M. Feorino, S. McDougal, D. Warfield, J. Getchell, C. Cabradilla, *et al*. (1986). The safety of the hepatitis B vaccine. Inactivation of the AIDS virus during routine vaccine manufacture. *JAMA* **256**: 869-872.

Galati, L., M. V. Chiantore, M. Marinaro, P. Di Bonito (2024). Human oncogenic viruses: characteristics and prevention strategies-lessons learned from human papillomaviruses. *Viruses* **16**: 416. doi: 10.3390/v16030416.

Gerlich, W. H. (2013). Medical virology of hepatitis B: how it began and where we are now. *Virol. J.* **10**: 239. doi: 10.1186/1743-422X-10-239.

Goodrum, G., M. Pelchat (2018). Insight into the contribution and disruption of host processes during HDV replication. *Viruses* **11**: 21. doi: 10.3390/v11010021.

Goto, K., A. A. Roca Suarez, F. Wrensch, T. F. Baumert, J. Lupberger (2020). Hepatitis C virus and hepatocellular carcinoma: When the Host Loses Its Grip. *Int. J. Mol. Sci.* **21**: 3057. doi: 10.3390/ijms21093057.

Grabar, S., D. Costagliola (2021). Epidemiology of Kaposi's sarcoma. *Cancers (Basel)* **13**: 5692. doi: 10.3390/cancers13225692.

Haider, M. B., A. Al Sbihi, A. J. Chaudhary, S. M. Haider, A. I. Edhi (2022). Hereditary hemochromatosis: Temporal trends, sociodemographic characteristics, and independent risk factor of hepatocellular cancer - nationwide population-based study. *World J. Hepatol.* **14**: 1804-1816. doi: 10.4254/wjh.v14.i9.1804.

Hajarizadeh, B., J. Grebely, G. J. Dore (2013). Epidemiology and natural history of HCV infection. *Nat. Rev. Gastroenterol. Hepatol.* **10**: 553-562. doi: 10.1038/nrgastro.2013.107.

Harding, A. T., N. S. Heaton (2022). The impact of estrogens and their receptors on immunity and inflammation during Infection. *Cancers (Basel)* **14**: 909. doi: 10.3390/cancers14040909.

Hartlage, A. S., A. Kapoor (2021). Hepatitis C virus vaccine research: Time to put up or shut up. *Viruses* **13**: 1596. doi: 10.3390/v13081596.

Hartley, C., P. Wasuwanich, T. Van, W. Karnsakul (2024). Hepatitis E vaccines updates. *Vaccines (Basel)* **12**: 722. doi: 10.3390/vaccines12070722.

Houghton, M. (2019). Hepatitis C virus: 30 years after its discovery. *Cold Spring Harb. Perspect Med.* **9**: a037069. doi: 10.1101/cshperspect.a037069.

IACR. Monographs, 2024, Volumes 1–136. https://monographs.iarc.who.int/list-of-classifications. Visited on 24.08.2024

Jang, T. Y., Y. J. Wei, T. W. Liu, M. L. Yeh, S. F. Liu, C. T Hsu *et al*. (2021). Role of hepatitis D virus infection in development of hepatocellular carcinoma among chronic hepatitis B patients treated with nucleotide/nucleoside analogues. *Sci. Rep.* **11**: 8184. https://doi.org/10.1038/s41598-021-87679-w.

Juang, H. H., C. W. Hsu, K. S. Chang, S. B. Iang, Y. H. Lin, M. Chao (2024). Investigating the genetic diversity of hepatitis delta virus in hepatocellular carcinoma (HCC): impact on viral evolution and oncogenesis in HCC. *Viruses* **16**: 817. doi: 10.3390/v16060817.

Kamal, H., G. Westman, K. Falconer, A. S. Duberg, O. Weiland, S. Haverinen et al. (2020). Long-term study of hepatitis Delta virus infection at secondary care centers: the impact of viremia on liver-related outcomes. *Hepatology*

72: 1177-1190. doi: 10.1002/hep.31214.

Kamar, N., J. Izopet, N. Pavio, R. Aggarwal, A. Labrique, H. Wedemeyer, H. R. Dalton (2017). Hepatitis E virus infection. *Nat. Rev. Dis. Primers* **3**: 17086. doi:10.1038/nrdp.2017.86.

Khuroo, M. S. (2011). Discovery of hepatitis E: the epidemic non-A, non-B hepatitis 30 years down the memory lane. *Virus. Res.* **161**: 3-14. doi: 10.1016/j.virusres.2011.02.007.

Kim, D. Y. (2024). Changing etiology and epidemiology of hepatocellular carcinoma: Asia and worldwide. *J. Liver Cancer* **24**: 62-70. doi: 10.17998/jlc.2024.03.13.

Klöhn, M., J. A. Schrader, Y. Brüggemann, D. Todt, E. Steinmann (2021). Beyond the usual suspects: hepatitis E virus and its implications in hepatocellular carcinoma. *Cancers* **13**: 5867. doi:10.3390/cancers13225867.

Kocarnik, J. M., M. May, A. Acheson, K. Bhangdia, K. Compton, F. Dean, W. Fu, L. Penberthy, N. Pritchett, R. Xu, N. Bhakta, L. Force (2024). The global burden of primary liver cancer and underlying etiologies from 1990 to 2021. *J.C.O.* **42**: 10573-10573. DOI:10.1200/JCO.2024.42.16_suppl.10573.

Komatsu, H., A. Inui, T. Fujisawa (2017). Pediatric hepatitis B treatment. *Ann. Transl. Med.* **5**: 37. doi: 10.2 1037/atm.2016.11.52.

Krump, N. A., J. You (2018). Molecular mechanisms of viral oncogenesis in humans. *Nat. Rev. Microbiol.* **16**: 684-698. doi: 10.1038/s41579-018-0064-6.

Le Gal, F., S. Brichler, T. Drugan, C. Alloui, D. Roulot, J. Pawlotsky, P. Dény, E. Gordien (2017). Genetic diversity and worldwide distribution of the *Deltavirus* genus: a study of 2,152 clinical strains. *Hepatology* **66**: 1826–1841. doi: 10.1002/hep.29574.

Lee, W., E. Langhoff. (2006). Polyomavirus in human cancer development. In: Ahsan, N. (Ed.) Polyomaviruses and Human Diseases. Advances in Experimental Medicine and Biology, vol 577. Springer, New York, NY. https://doi.org/10.1007/0-387-32957-9_22.

Lewis, D. J., J. F. Sobanko, J. R. Etzkorn, T. M. Shin, C. N. Giordano, S. L. McMurray, J. L. Walker, J. Zhang, C. J. Miller, H. W. Higgins 2nd (2023). Merkel cell carcinoma. *Dermatol. Clin.* **41**: 101-115. doi: 10.1016/j.det.2022.07.015.

Li, Y. T., H. L. Wu, C. J. Liu (2021). Molecular mechanisms and animal models of HBV-related hepatocellular Carcinoma: with emphasis on metastatic tumor antigen 1. *Int. J. Mol. Sci.* **22**: 9380. doi: 10.3390/ijms22179380.

Liang, Y. J. C. P. Sun, Y. C. Hsu, Y. W. Chen, I. A. Wang, C. W. Su, M. H. Tao, J. C. Wu (2020). Statin inhibits large hepatitis delta antigen-Smad3-twist-mediated epithelial-to-mesenchymal transition and hepatitis D virus secretion. *J. Biomed. Sci.* **27**: 65. doi: 10.1186/s12929-020-00659-6.

Liu, Z., Y. Zhang, M. Xu, X. Li, Z. Zhang (2021). Distribution of hepatitis B virus genotypes and subgenotypes: A meta-analysis. *Medicine (Baltimore)* **100**: e27941. doi: 10.1097/MD.0000000000027941.

Llovet, J. M., Kelley, R. K., A. Villanueva, A. G. Singal, E. Pikarsky, S. Roayaie, R. Lencioni, K. Koike, J. Zucman-Rossi, R. S. Finn (2021). Hepatocellular carcinoma. *Nat. Rev. Dis. Primers*. **7**: 6. https://doi.org/10.1038/s41572-020-00240-3

Lombardo, D., M. S. Franzè, G. Caminiti, T. Pollicino T. (2024). Hepatitis Delta virus and hepatocellular carcinoma. *Pathogens* **13**: 362. doi: 10.3390/pathogens13050362.

Ma, W. L., H. C. Lai, S. Yeh, X. Cai, C. Chang (2014). Androgen receptor roles in hepatocellular carcinoma, fatty liver, cirrhosis and hepatitis. *Endocr. Relat. Cancer.* **21**: R165-82. doi: 10.1530/ERC-13-0283.

Malaina, I., L. Martinez, D. Salcines-Cuevas, H. Teran-Navarro, J.G. Ocejo-Vinyals, E. Gonzalez-Lopez, V. Soriano, M. Ubeda, M. B. Perez Pinilla, I. Martinez de la Fuente, C. Alvarez-Dominguez (2023). Testing a vaccine candidate against Hepatitis C virus designed by combinatorial optimization. *Sci. Rep*. **13**: 21746. https://doi.org/10.1038/s41598-023-48458-x

Manne, V., J. Ryan, J. Wong, G. Vengayil, S. A. Basit, R. G. Gish (2021). Hepatitis C vaccination: where we are and where we need to be. *Pathogens* **10**: 1619. doi: 10.3390/pathogens10121619.

McAleer, W. J., E. B. Buynak, R. Z. Maigetter, D. E. Wampler, W. J. Miller, M. R. Hilleman (1984). Human hepatitis B vaccine from recombinant yeast. *Nature* **307**: 178-80. doi: 10.1038/307178a0.

Mesri, E. A., M. A. Feitelson, K. Munger (2014). Human viral oncogenesis: a cancer hallmarks analysis. *Cell Host Microbe* **15**: 266-82. doi: 10.1016/j.chom.2014.02.011.

Miao, Z., S. Zhang, X. Ou, S. Li, Z. Ma, W. Wang, M. P. Peppelenbosch, J. Liu, Q. Pan (2020). Estimating the global prevalence, disease progression, and clinical outcome of hepatitis Delta virus infection. *J. Infect. Dis.* **221**: 1677-1687. doi: 10.1093/infdis/jiz633.

Mironova, M., M. G. Ghany (2024). Hepatitis B vaccine: four decades on. *Vaccines (Basel)* **12**: 439. doi: 10.3390/vaccines12040439.

Negro, F., A. S. Lok (2023). Hepatitis D: a review. *JAMA*. **330**: 2376-2387. doi:10.1001/jama.2023.23242.

Nesbitt, R. C., V. K. Asilaza, E. Gignoux, A. Koyuncu, P. Gitahi, P. Nkemenang, *et al*. (2024). Vaccination coverage and adverse events following a reactive vaccination campaign against hepatitis E in Bentiu displaced persons camp, South Sudan. *PLoS Negl. Trop. Dis*. 18: e0011661. doi: 10.1371/journal.pntd.0011661.

Ng, J., J. Wu (2012). Hepatitis B- and hepatitis C-related hepatocellular carcinomas in the United States: similarities and differences. *Hepat. Mon*. **12**: e7635. doi: 10.5812/hepatmon.7635.

Omar, A., N. Marques, N. Crawford (2024). Cancer and HIV: The molecular mechanisms of the deadly duo. *Cancers (Basel)* **16**: 546. doi: 10.3390/cancers16030546.

Omar, M. A., M. M. Omran, K. Farid, A. A. Tabll, Y. E. Shahein, T. M. Emran, A. Petrovic, N. R. Lucic, R. Smolic, T. Kovac, M. Smolic (2023). Biomarkers for Hepatocellular Carcinoma: From Origin to Clinical Diagnosis. *Biomedicines* **11**: 1852. doi: 10.3390/biomedicines11071852.

Parikh, N. D., A. S. Mehta, A. G. Singal, T. Block, J. A. Marrero, A. S. Lok (2020). Biomarkers for the early detection of hepatocellular carcinoma. *Cancer Epidemiol. Biomarkers Prev*. **29**: 2495-2503. doi: 10.1158/1055-9965.EPI-20-0005.

Plummer, M., C. de Martel, J. Vignat, J. Ferlay, F. Bray, S. Franceschi (2016). Global burden of cancers attributable to infections in 2012: a synthetic analysis. *Lancet Glob. Health*. **4**: e609-16. doi: 10.1016/S2214-109X(16)30143-7.

Pol., S, A. Vallet-Pichard, O. Hermine (2018). Extrahepatic cancers and chronic HCV infection. *Nat. Rev. Gastroenter-*

ol. Hepatol. **15**: 283-290. doi: 10.1038/nrgastro.2017.172.

Prado, J. C. M., T. A. Monezi, A.T. Amorim, V. Lino, A. Paladino, E. Boccardo (2018). Human polyomaviruses and cancer: an overview. *Clinics (Sao Paulo)*. **73**: e558s. doi: 10.6061/clinics/2018/e558s.

Puigvehí, M., C. Moctezuma-Velázquez, A. Villanueva, J.M. (2019). The oncogenic role of hepatitis delta virus in hepatocellular carcinoma. *JHEP Rep*. **1**: 120-130. doi: 10.1016/j.jhepr.2019.05.001.

Reungoat, E., B. Grigorov, F. Zoulim, E. I. Pécheur (2021). Molecular Crosstalk between the Hepatitis C Virus and the Extracellular Matrix in Liver Fibrogenesis and Early Carcinogenesis. *Cancers (Basel)* **13**: 2270. doi: 10.3390/cancers13092270.

Rizzetto, M. (2015). Hepatitis D virus: introduction and epidemiology. *Cold Spring Harb. Perspect. Med.* **5**7: a021576. doi: 10.1101/cshperspect.a021576.

Rizzetto, M., A. Ciancio (2012). Epidemiology of hepatitis D. *Semin Liver Dis*. **32**: 211-219. doi: 10.1055/s-0032-1323626.

Rizzetto, M., M. G. Canese, S. Aricò, O. Crivelli, C. Trepo, F. Bonino, G. Verme (1977). Immunofluorescence detection of new antigen-antibody system (delta/anti-delta) associated to hepatitis B virus in liver and in serum of HBsAg carriers. *Gut* **18**: 997-1003. doi: 10.1136/gut.18.12.997.

Rizzetto, M., S. Hamid, F. Negro (2021). The changing context of hepatitis D. *J. Hepatol.* **74**: 1200-1211. doi: 10.1016/j.jhep.2021.01.014.

Rizzetto, M., S. M. Alavian (2013). Hepatitis delta: the rediscovery. *Clin. Liver Dis.* **17**: 475-87. doi: 10.1016/j.cld.2013.05.007.

Romano, L., A. R. Zanetti (2022). Hepatitis B vaccination: a historical overview with a focus on the Italian achievements. *Viruses* **14**: 1515. doi: 10.3390/v14071515.

Rumgay, H., M. Arnold, J. Ferlay, O. Lesi, C. J. Cabasag, J. Vignat, M. Laversanne, K. A. McGlynn, I. Soerjomataram (2022). Global burden of primary liver cancer in 2020 and predictions to 2040. *J. Hepatol.* **77**: 1598-1606. doi: 10.1016/j.jhep.2022.08.021.

Sapena, V., M. Enea, F. Torres, C. Celsa, J. Rios, G.E.M. Rizzo, P. Nahon, Z. Mariño, R. Tateishi, T. Minami (2022). Hepatocellular carcinoma recurrence after direct-acting antiviral therapy: an individual patient data meta-analysis. *Gut* **71**: 593-604. doi: 10.1136/gutjnl-2020-323663.

Schiller, J. T., D. R. Lowy (2021). An introduction to virus infections and human cancer. *Recent Results Cancer Res*. **217**: 1-11. doi: 10.1007/978-3-030-57362-1_1.

Shi, L., Y. Feng, H. Lin, R. Ma, X. Cai (2014). Role of estrogen in hepatocellular carcinoma: is inflammation the key? *J. Transl. Med*. **12**: 93. https://doi.org/10.1186/1479-5876-12-93

Smedile, A., Verme, G. (1999). Hepatitis D Virus. In: Specter, S. (Ed). Viral Hepatitis. Humana Press, Totowa, NJ. https://doi.org/10.1007/978-1-59259-702-4_5.

Stockdale, A. J., B. Kreuels, M. Y. R. Henrion, E. Giorgi, I. Kyomuhangi, C. de Martel, Y. Hutin, A. M. Geretti (2020). The global prevalence of hepatitis D virus infection: Systematic review and meta-analysis. *J. Hepatol.* **73**: 523-532. doi: 10.1016/j.jhep.2020.04.008.

Szmuness, W., C. E. Stevens, E. J. Harley, E. A. Zang, W. R. Oleszko, D. C. William, R. Sadovsky, J. M. Morrison, A. Kellner (1980). Hepatitis B vaccine: demonstration of efficacy in a controlled clinical trial in a high-risk population in the United States. *N. Engl. J. Med.* **303**: 833-841. doi: 10.1056/NEJM198010093031501.

Talamantes, S., M. Lisjak, E. H. Gilglioni, C. J. Llamoza-Torres, B. Ramos-Molina, E. N. Gurzov (2023). Non-alcoholic fatty liver disease and diabetes mellitus as growing aetiologies of hepatocellular carcinoma. *JHEP Rep*. **5**: 100811. doi: 10.1016/j.jhepr.2023.100811.

Tamura, I., O. Kurimura, T. Koda, H. Ichimura, S. Katayama, T. Kurimura, Y. Inaba (1993). Risk of liver cirrhosis and hepatocellular carcinoma in subjects with hepatitis B and delta virus infection: a study from Kure, Japan. *J. Gastroenterol. Hepatol*. **8**: 433-436. doi: 10.1111/j.1440-1746.1993.tb01543.x.

The Lancet Gastroenterology Hepatology (2021). The hunt for a vaccine for hepatitis C virus continues. Lancet Gastroenterol. *Hepatol*. **6**: 253. doi: 10.1016/S2468-1253(21)00073-X.

Toyé, R. M., C. L. Loureiro, R. C. Jaspe, F. Zoulim, F. H. Pujol, I. Chemin (2023). The Hepatitis B Virus Genotypes E to J: The Overlooked Genotypes. *Microorganisms* **11**: 1908. doi: 10.3390/microorganisms11081908.

Tsukiyama-Kohara, K., M. Kohara (2017). Hepatitis C Virus: Viral quasispecies and genotypes. *Int. J. Mol. Sci*. **19**: 23. doi: 10.3390/ijms19010023.

Villanueva, A. (2019). Hepatocellular Carcinoma. *N. Engl. J. Med.* **380**: 1450–1462. doi: 10.1056/NEJMra1713263.

Wang, W., C. Wei (2020). Advances in the early diagnosis of hepatocellular carcinoma. *Genes Dis*. **7**: 308-319. doi: 10.1016/j.gendis.2020.01.014.

Waziry, R., B. Hajarizadeh, J. Grebely, J. Amin, M. Law, Danta M, J. George, G. J. Dore (2017). Hepatocellular carcinoma risk following direct-acting antiviral HCV therapy: A systematic review, meta-analyses, and meta-regression. *J. Hepatol.* **67**: 1204-1212. doi: 10.1016/j.jhep.2017.07.025.

World Health Organization (2017). Global Hepatitis Report. doi:ISBN 978-92-4-156545-5.

Yang, F., L. Ma, Y. Yang, W. Liu, J. Zhao, X. Chen, M. *et al*. (2019). Contribution of hepatitis B virus infection to the aggressiveness of primary liver cancer: a clinical epidemiological study in Eastern China. *Front. Oncol*. **9**: 370. doi: 10.3389/fonc.2019.00370.

Yin, X., F. Kan (2023). Hepatitis E virus infection and risk of hepatocellular carcinoma: A systematic review and meta-analysis. *Cancer Epidemiol*. **87**: 102457. doi: 10.1016/j.canep.2023.102457.

Zella, D., R. C. Gallo (2021). Viruses and bacteria associated with cancer: an overview. *Viruses* **13**: 1039. doi: 10.3390/v13061039.

Zhang, J., X. F. Zhang, S. J. Huang, T. Wu, Y.M. Hu, Z. Z. Wang, H. *et al*. (2015). Long-term efficacy of a hepatitis E vaccine. *N. Engl. J. Med*. **372**: 914-922. doi: 10.1056/NEJMoa1406011.

Zhang, Z. H., C. C. Wu, X. W. Chen, X. Li, J. Li, M.J. Lu (2016). Genetic variation of hepatitis B virus and its significance for pathogenesis. *World J. Gastroenterol*. **22**: 126-144. doi: 10.3748/wjg.v22.i1.126.

zur Hausen, H. (2009) The search for infectious causes of human cancers: where and why (Nobel lecture). *Angew Chem. Int. Ed. Engl*. **48:** 5798-5808. doi: 10.1002/anie.200901917.