Universidade de Lisboa

Faculdade de Farmácia

New drugs for the treatment of liver diseases

Advances on treatments for hepatocellular carcinoma

Simaura Alexandra Marques Faria

Mestrado Integrado em Ciências Farmacêuticas

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Resumo

As doenças hepáticas afetam milhões de pessoas todos os anos, tanto em países industrializados como países em desenvolvimento, podendo ter diferentes causas, tais como vírus, distúrbios metabólicos, álcool e alterações imunológicas. A principal consequência de doença hepática em estado avançado é a cirrose, que habitualmente progride para carcinoma hepatocelular.

Apesar da descoberta da vacina do vírus da Hepatite B ter causado um grande impacto na redução da dimensão das doenças hepáticas, bem como na incidência do carcinoma hepatocelular, não foi uma solução, visto que apenas se trata de uma das possíveis causas de doença hepática, e ainda existe população não vacinada.

Para além disso, o aumento de distúrbios metabólicos, tais como obesidade e diabetes, nos países desenvolvidos, que são maioritariamente causados pela dieta hipercalórica ocidental, leva ao aumento do número de pacientes com cirrose e, em última instância, com carcinoma hepatocelular.

O diagnóstico do carcinoma hepatocelular, atualmente realizado através de técnicas de imagem e por determinação dos níveis séricos de enzimas hepáticas, deve ser efetuado o mais cedo possível, de modo a permitir um bom prognóstico e a possibilidade de transplante, que é atualmente o único tratamento curativo. Programas de rastreio estão em falta na maioria dos países e é algo que precisa de ser resolvido, visto ser uma opção de baixos custos e recursos para diminuir a globalidade de casos de carcinoma hepatocelular.

Para estados iniciais de carcinoma hepatocelular, as opções terapêuticas são maioritariamente cirúrgicas, incluindo ressecção e transplante hepático, apesar de existir também alguma farmacologia. O primeiro fármaco sistémico aprovado foi o sorafenib, em 2008, e apesar dos esforços e constante investigação, apenas 10 anos depois foram descobertos novos fármacos eficazes.

Para melhor perceber o desenvolvimento, diagnóstico e tratamento do carcinoma hepatocelular, começar-se-á por introduzir o fígado: células hepáticas, principais funções e a importância no organismo humano; de seguida serão descritas as doenças hepáticas mais comuns. Por último, o foco centrar-se-á no carcinoma hepatocelular,

referindo a incidência, fisiopatologia, atuais opções terapêuticas, mas também opções promissoras em desenvolvimento.

Palavras-chave: Doenças hepáticas; carcinoma hepatocelular; diagnóstico; terapêutica; investigação

Abstract

Liver diseases affect millions of people every year, both in industrialized and developing countries, having several different etiologies, such as virus, metabolic disorders, alcohol and immunological disorder. Cirrhosis is the primary consequence of advanced liver disease, which commonly progresses to hepatocellular carcinoma.

Although the Hepatitis B Virus vaccine discovery had a great impact in reducing liver disease burden, and also hepatocellular carcinoma incidence, it was not a full solution, as the Hepatitis B Virus is only one of the possible causes of liver diseases, and there are still many unvaccinated people.

The worldwide increase of metabolic disorders, such as obesity and diabetes, mainly due to the hypercaloric western diet, is leading to the rise of cirrhosis and, consequently, hepatocellular carcinoma.

The diagnosis of hepatocellular carcinoma, which is currently approached through imaging techniques and measurement of serum levels of specific hepatic enzymes, should be perform as early as possible, to allow for a better prognosis and the possibility of liver transplant, which currently is the only curative treatment option. Screening programs are lacking in most countries and this needs to be addressed, since it constitutes a low-cost and low-resource tool to decrease hepatocellular carcinoma global burden.

Treatment options for hepatocellular carcinoma in early stages are mostly surgical, including liver resection and transplant, but different drugs are also available. The first approved systemic drug for hepatocellular carcinoma was sorafenib, in 2008, and despite the efforts and constant research, only 10 years later novel drugs were discovered.

To better understand development, diagnosis and treatment of hepatocellular carcinoma, we will start with an overview of the liver: different types of hepatic cells and its role in the human organism; we will then describe the most common liver diseases. Last but not least, we will focus on hepatocellular carcinoma, referring to incidence, physiopathology, current treatment options, but also on-going research and novel therapeutics approaches.

Keywords: Liver diseases, hepatocellular carcinoma; diagnosis; therapy; research

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Abbreviations

AASLD	American Association for the Study of Liver Diseases
AFP	Alpha-Fetoprotein
ALT	Alanine-Amino-Transferase
AST	Aspartate-Amino-Transferase
BCLC	Barcelona Clinic Liver Cancer
CIK	Cytokine-Induced Killer
CLDs	Chronic Liver Diseases
CT	Computed Tomography
CTP	Child-Turcotte-Pugh
DCP	Des-γcarboxiprothrombin
EASL	European Association for the Study of the Liver
EGFR	Epidermal Growth Factor Receptor
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HGDNs	High Grade Dysplastic Nodules
HSC	Hepatic Stellate Cells
HSV	Herpes Simplex Virus
IgM	Immunoglobuline M
LGDNs	Low Grade Dysplastic Nodules
LSEC	Liver Sinusoidal Endothelial Cells
MWA	Microwave Ablation
NAFLD	Non-Alcoholic Fatty Liver Disease
NASH	Non-Alcoholic Steatohepatitis
OS	Overall Survival

OV	Oncolytic Virus
OVT	Oncolytic Virotherapy
PBT	Proton Beam Therapy
PPAR	Peroxisomal Proliferator Activated Receptor
PS	Performance Scale
RFA	Radiofrequency Ablation
TACE	Transartherial Chemoembolization
TARE	Transartherial Radioembolization
TERT	Telomere Reverse Transcriptase
TIL	Tumor Infiltrating Lymphocyte
VEGFR	Vascular Endothelial Growth Factor Receptor

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1. Introduction

Hepatocellular carcinoma (HCC) is on the top 10 list of the most commonly diagnosed cancers, being mainly detected in the late stages(1). The American Cancer Society (ACS) estimates 42810 new cases for 2020 in the United States of America (USA), of which 30160 will result in death (2).

China is the country with the highest incidence of HCC, and Asia is estimated to have around 70% of all the cases of HCC worldwide (1), followed by Africa (3). However, in Portugal, liver malignancy is not one of the most common tumors, according to "Portugal- Doenças Oncológicas em números", published in 2015 (4).

HCC incidence closely relates to others liver pathologies, with the main risk factors for HCC being Hepatitis C virus (HCV); Hepatitis B Virus (HBV); heavy alcohol drinking and diabetes (5). As a result, HCC incidence and mortality rates have been decreasing in the traditionally high-risk regions, such as Japan and parts of China, due to public health efforts in reducing incidence and transmission of HCV and HBV (3).

Throughout this work, we will address and discuss liver physiopathology, focusing on hepatocellular carcinoma. In this regard, we will further list current and promising therapeutic approaches for this deadly disease.

2. Goals

The main goal of this work is to review and summarize the pathophysiology of hepatocellular carcinoma, currently available treatments and recent discoveries in terms of novel therapeutic options. As background, the functions and structure of the liver, as well as its most common diseases will be presented, conducting to the relevance of hepatocellular carcinoma as one of the deadliest liver diseases and the importance of discovering novel treatments to cure liver cancer.

3. Methods

Queries for original and review research papers was performed using mostly the *PubMed* database, and choosing the most recent articles, to assure that information was up-to-date. At first, around 40 articles were downloaded and analyzed, and new queries were performed when needed.

4. The Liver

The liver is the biggest internal organ in the human body, accounting for 2 to 5% of total body weight. (6) It is located in the upper right quadrant of the abdomen, with a small part in the upper left quadrant.

4.1 Liver Functions

The liver has over 500 functions, being primarily known for acting as a filter of the human body where xenobiotics, and other harmful foreign molecules, suffer biotransformation and are metabolized (7).

The liver is responsible for many physiological processes: it performs metabolic functions, such as storing glucose in the form of glycogen, later providing it to the brain as glucose and to the muscles as acetoacetate; synthetic functions, playing a major role in the processing of proteins and being responsible for the synthesis of most plasma proteins; it is involved in the metabolism of amino acids and also the synthesis of blood coagulation factors and some immunologic functions, protecting the body from foreign molecules through its metabolization (8-10). Additionally, the liver oxidizes lipids, being involved in the cholesterol uptake; maintains the plasmatic concentration of many organic and inorganic components and is also involved in blood volume regulation (8, 9).

Another essential function of the liver is bile formation. The hepatocytes produce bile acids, resulting from the hepatic metabolization of cholesterol. Bile acids are necessary not only for digestion and nutrient absorption (lipids, fat-soluble vitamins) but for metabolizing lipophilic toxins and xenobiotics. After synthesis, bile acids are transported into the canalicular space, also called bile canaliculi- the space between two adjacent hepatocytes, that takes the bile into a series of larger tubes (11, 12).

4.2 Liver Structure

Conventionally, the liver is divided into four lobes: right, left, quadrate and caudate, with the ligamentum falciform partitioning the right and left anatomic lobes. The caudate lobe is sometimes considered a separated part of the liver, because it’s features an independent blood supply and biliary drainage (7, 13).

This classical division of the liver does not account for the internal vessels and duct branchings, which are of great importance. Through the years, many terminologies and

divisions have been suggested, and although different health care professionals still tend to use different terminologies, the Federative Committee on Anatomical Terminology (FCAT) created an internationally accepted one, in which the liver is divided into seven segments (13).

If we sectioned the liver in many different angles, we would observe that it has the same basic histologic appearance: multiple units with a central hepatic vein (hepatic venule). (7) Based on this, the liver can be functionally divided into three microscopic units that are called lobules, liver zonation (Table 1) (12).

Table 1 Liver zonation (12)

Zone	Placing	Function
1	Close to portal tracts	Gluconeogenesis; urea production and β-oxidation of fatty acids
2	Between the portal tracts and central veins	Contact with oxygen—intermediate and potentially toxin-intermediate blood.
3	Close to the central veins	Glycolysis; use of glutamine synthetase to remove nitrogen and engage in lipogenesis

The liver blood flow corresponds to one-quarter (25% to 30%) of the heart output at rest. There are two distinct hepatic blood supplies: the portal vein, responsible for about 70% of the blood flow and 40% of the oxygen; and the hepatic artery, responsible for 60% of the oxygen and 30% of the blood flow (7, 9).

The portal blood drains the venous return from the splenic, mesenteric, gastric and pancreatic arteries, receiving all digested and absorbed substances. This forms the first pass metabolism, which prevents organism intoxication. Blood flow, both venous and arterial, mix together in the liver capillaries- sinusoids- and are later emptied into terminal hepatic venules and other veins, until it reaches the vena cava (7, 11, 12).

4.3 Liver Anatomy

There are at least 15 different cell types in the liver (7). These cell types can be divided into two major kinds of cell: parenchymal and non-parenchymal cells. Hepatocytes are the parenchymal cells of the liver and the non-parenchymal cells include liver sinusoidal endothelial cells (LSECs), hepatic stellate cells (HSCs) and liver-specific immune cells, called Kupffer cells (11).

Hepatocytes are the major liver cell, accounting for about 60% of all hepatic cells and 80% of liver volume. They are responsible for the majority of liver functions, contain numerous mitochondria and are considered cellular factories (6, 12). Hepatocytes are organized in plates, 1 cell thick, placed side to side with hepatic sinusoids, creating a bigger surface area of contact between the hepatocytes and the blood that flows in the sinusoids (12).

The main function of hepatocytes is to produce bile (about 15ml/kg/day in humans), being the only cell type in the human body capable of bile acids synthesis from *de novo* synthesis from cholesterol (7, 11). Hepatocytes are also responsible for the synthesis and secretion of plasma proteins and lipoproteins, including albumin, coagulation factors and acute phase proteins; are essential to detoxification, expressing a large variability of detoxifying enzymes -generally called P450 enzymes- that identify and eliminate harmful molecules through bile or urine; and exhibit other metabolic functions, such as glutamine synthesis, urea formation, gluconeogenesis and metabolizing and storing of nutrients (11, 12).

LSECs are the cells lying between blood and hepatocytes, accounting for about 20% of liver cells. These cells differ from others phenotypically and have unique morphological characteristics (7, 14). Their main function is to filter fluids, solutes and particles through fenestrae- small openings that can transfer molecules from sinusoidal space into the space of Disse by endocytosis. If the fenestrae suffers changes, in its diameter or frequency, there can be a higher risk of diseases due to loss of liver functions. For example, if there is less fenestrae, less cholesterol is removed from circulation and accumulates in blood, creating a higher risk for atherosclerosis (7, 14).

LSECs have other functions such as immune regulation; maintaining liver capillaries hemodynamics and responding to variations on hepatic blood flow and pressure. LSECs play an active role in controlling hepatocytes maintenance and regeneration, making LSECs-hepatocyte communication a vital factor in liver function, both on a healthy and fibrotic liver (14).

HSCs correspond to about 5% of liver cells. They are considered fat-storing cells, and their primary role is to produce extracellular matrix (ECM), which regulates and modulates hepatic function. HSCs control microvascular tone and store and metabolize

vitamin A and lipids. HSCs are in straight communication with LSECs, which is vital to the correct function of LSECs (7, 14).

As many other organs, the liver possesses a specific innate immune system. This consists mostly of Kupffer cells, the liver macrophages that account for about 15% of the liver cells. These cells proliferate locally, are characterized as phagocytic and produce large amounts of cytokines and reactive oxygen species that act as inflammation mediators, making them vital to the recognition and elimination of foreign materials in the portal circulation (7, 11, 14).

Kupffer cells prevent many immune reactions by blocking the access of bacteria and food antigens to the systemic blood (9). In the presence of damage caused by alcohol. Kupffer cells are activated, as is the rest of the innate immune system, producing tumor necrosis factor (TNF) that will cause parenchymal stress response (11, 14).

5. Liver diseases

Although liver diseases are often asymptomatic, there are approximately 844 million people in the world with a type of chronic liver disease (CLD), accounting for almost 2 million deaths every year, minus accounting for acute injuries. As previously mentioned the liver has important functions in the detoxification of the human organism (15, 16).

In parallel with detoxification, the liver performs a whole range of different functions and there's still no therapeutic strategies that can manage all of them in case of damage, making liver transplantation the only permanent curative solution. This lack of therapeutic options requires an urgent solution, and it is crucial to develop investigations and research on novel treatments, dependent on each etiology of the condition (6).

There are several causes for liver diseases: toxins, viruses, biliary obstruction, metabolic, immune and vascular problems.

Various substances get into the liver through intestinal absorption and subsequent transportation through the portal vein, undergoing first-pass metabolism. Some of these substances are toxic to the liver and this can occur by different mechanisms. Hepatotoxins directly damage hepatocytes, while other toxins only become toxic due to enzymatic modification through cytochrome P450 enzymes, most common in zone

3 hepatocytes. Both of these types of toxins can cause either acute or chronic damage (12).

Damage caused by viruses are mostly attributed to hepatitis viruses, which have specific liver interactions, while biliary obstruction also causes liver disease and further leads to bile acids accumulation, that can be toxic when at high concentrations and/or lead to the formation of gallstones (12).

Liver damage can also be caused by fat accumulation, or steatosis, clots, hyperimmunity and immune incompatibility (12).

Liver damage usually leads to hepatocyte death, which causes different changes in the liver in order to adapt cellular mechanisms. These changes may occur in an acute or chronic manner.

Acute liver damage usually occurs in a short period of time and does not evolve to permanent damage. Still, it can cause so much hepatocyte loss, which can lead to acute liver failure (12).

In turn, CLDs represent a serious health problem worldwide, underestimated and with no national or international health programs or public awareness. This happens because CLD does not exhibit symptoms until the late stages, being considered a silent disease. Even physicians have trouble in its diagnosis because it presents normal clinical presentation and biochemical testing is still very limited (15).

CLDs are diseases that develop over several years, with the main underlying process in this progression being liver inflammation. This process can lead, in the first stages, to liver dysfunction. In addition, hepatocyte inflammation can cause necrosis, through modulation of immune responses, a process known as necroinflammation. Necroinflammation can advance to fibrosis or even cirrhosis, leading to HCC (Figure 1) (12, 15).

It has been shown that the necroinflammation grade relates to the stage of liver fibrosis, which is used in evaluating CLD. This measure is used in determining the state of the disease, differencing “chronic active hepatitis” from “chronic persistent hepatitis”, and can also be used in the prognosis of the CLDs. There is evidence that stopping inflammation, with for instance retroviral therapy targeting HBV or HCV, also halts fibrogenesis and can even regress fibrosis, through natural fibrolysis. As a result, liver inflammation is a main focus of liver therapy development (15).

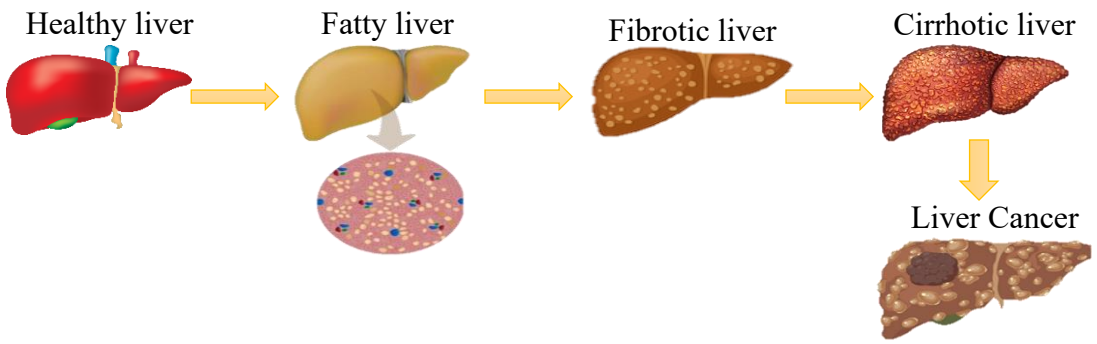

Figure 1 Distinct phases of liver disease that may lead to HCC. Chronic liver diseases starts with hepatocytes damage or death, followed by inflammation and fibrosis. Later, it can progress to serious complications, such as cirrhosis and HCC, which can lead to death.

Figure adapted from (17).

5.1 Hepatitis

Five different hepatitis viruses have been identified so far, namely Hepatitis A virus (HAV), Hepatitis B Virus (HBV), Hepatitis D Virus (HDV) and Hepatitis E Virus (HEV).

Hepatitis A virus (HAV) was first isolated in 1979, characterized as a ribonucleic acid (RNA) small virus, non-enveloped but very resistant. It can tolerate pH as low as one, which allows it to survive in the human stomach, and is also tolerant to high temperatures, being transmitted in food that is not cooked thoroughly (at least 1 minute at 85ºC) (18).

The HAV vaccine was first commercialized in 1995, drastically reducing the cases numbers. Previous to this, the HAV had a high transmission rate, through fecal-oral via and due to the lack of proper hygiene, being humans his only host. The viruses particles reach the stomach, are not destroyed, and pass to the liver through the portal circulation, where they replicate inside the hepatocytes (18).

Clinical evaluation of HAV infection is not straightforward. It is usually performed on unvaccinated people or those with high-risk behaviors and that exhibit symptoms, namely fatigue, fever, vomiting, nausea and jaundice. The adult population is more commonly symptomatic, presenting jaundice in 70% of the cases. Laboratory tests usually depict an elevation of specific liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT), bilirubin and alkaline phosphatase. Confirmation

of diagnosis is made through a positive serologic test for HAV immunoglobulin M (IgM), that can become positive after 5 days of illness or after vaccination (18).

HAV treatment is limited to supportive care, controlling the symptoms of the disease and preventing transmission, with proper hygiene. Liver failure in HAV patients is extremely rare. In fact, HAV infection rarely evolves to a chronic state, for which surveillance screening is not usually recommended, although contamination in poor sanitation areas are still common (18).

HBV is the smallest desoxyribonucleic acid (DNA) enveloped virus identified. The highest number of HBV infection concentrates in Asia and sub-Saharan Africa, due to lack of vaccination (16, 18).

Table 2 Hepatitis B laboratory values (18)

Name	Abbreviation	Positive value indicates	Meaning
Hepatitis B surface antibody	Anti-HB	Present immunity to HBV (through vaccination or natural exposition)	Past immunization or past infection
Hepatitis B core antibody	Anti-HBc	Acute, chronic or cleared infection	Exposition to natural HBV
Hepatitis B surface antigen	HBsAg	Patient currently infected and infectous	Hepatitis B status evaluation
Hepatitis B core antibody IgM	IgM anti-HBc	Acute infection	Determine if chronic (if HBsAg positive) or acute infection

HBV shares a very similar infection mode with HAV, displaying similar symptoms-also commonly asymptomatic- and being diagnosed through laboratory tests: elevated ALT and AST levels, with normal bilirubin levels. An immunologic evaluation (Table 2) confirms HBV infection. However, and unlike HAV, acute HBV infection can progress to a chronic stage causing long-term liver changes, which are not usually severe except in individuals over 60 years old, and the fatality rate is very low (18).

HBV is transmitted through body fluids (semen, blood, vaginal fluids) usually through needles sharing, sexual contact, during birth or an open wound. It enters the circulation until it gets to the liver and replicates in hepatocytes, altering their functions. (18)

HCV infection has a great impact worldwide, with more or less 200 million infections per years, causing around 700 000 deaths. HCV infections are predominant in Asia and sub-Saharan Africa, and the great majority of acute infections evolve to chronic infections (16, 19).

Although HCV was identified in the late eighties, it still represents a major cause for liver transplant, due to mostly asymptomatic cases leading to advanced stage diagnosis. HCV screening programs in risk patients, which would allow for earlier diagnosis, are lacking (16, 19).

HCV is classified as an RNA enveloped virus with 11 known genotypes. The virus particles cause an immunologic response when they infiltrate hepatocytes, activating CD4+ T lymphocytes and leading to lysis of hepatic cells. The virus is transmitted through percutaneous exposure, such as sharing of needles (19).

There are different diagnostic tests available for HCV infection. The first one, also used for screening high risk patients, is a serologic test for anti-HCV antibodies. Routine laboratory tests can also be performed, usually looking for high levels of ALT. Confirmation of diagnosis is performed by checking for viral RNA in a serum or plasma sample. After diagnosis confirmation, a liver biopsy is commonly needed to assess liver fibrosis status and HCV stage. Alternatively, ultrasound imaging can be used, although it only accurately detects disease stage 4 and can lead to false results in obese patients (19).

HCV staging is crucial to decide treatment. The METAVIR or the Knodell scoring system can be used. There are currently two different treatment pathways: the first one targets the immune response, trying to stop it and slow viral spread and the second one targets the viral particles altering the viral replication and maturation (19).

Another essential determination to decide the course of treatment is to determine the HCV genotype, allowing an adequate drug choice, dosage and treatment duration. The more common genotypes are 1 to 6 (19).

Hepatitis D virus (HDV) can only infect an individual if he carries the hepatitis B surface antigen (HBsAg), causing the HBV infection to aggravate. It’s considered the

unhealthiest type of viral hepatitis in humans, speeding the process of cirrhosis and causing earlier irreversible damage in liver functions (20).

To diagnose HDV, a serologic test should be performed in order to detected antibodies to the hepatitis delta (HD) antigen (anti-HD), recognizing only current infections. Diagnosis needs to be confirmed through a prove of superinfected HBsAg with HDV (20).

In Europe, HDV has been an emerging health problem, mostly due to immigration from zones where HDV is still a pandemic problem (20).

Hepatitis E Virus (HEV) is transmitted zoonotically, through contaminated water or food or direct contact with infected animals. It is considered to be endemic in various industrialized zones, including in Europe, but also in poorer areas (21).

HEV is a small RNA virus, single-stranded and with an icosahedral capsid. There are two types of infections particles: unenveloped virions, found in feces; and quasi-enveloped particles, found in blood. There are four genotypes of HEV that can infect and reproduce itself on humans, the predominant one being genotype 3 (21).

HEV infection is similar to other viral hepatitis and only immunocompromised patients usually develop chronic infections. The clinical manifestations of HEV can include neurological alterations, neuropathic pain, meningitis, encephalitis and other brain-related injuries. HEV viral infection is typically detected through measurement of immunological biomarkers, anti-HEV IgM and immunoglobulin G (IgG); or through direct search of RNA or HEV antigen in blood and feces (21).

5.2 Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease nowadays, due to the continuous increase in worldwide obesity. It is estimated that 20 to 30% of all adults have excess fat in the liver, which makes for a high percentage of global NAFLD incidence. The world is not yet prepared for this burden, lacking health awareness and screening programs or even strategic therapeutics. Surprisingly, NAFLD is not the liver disease with the highest mortality rate, but this can be explained by its correlation with other metabolic problems and cardiovascular consequences, which cause severe health problems (15, 16, 22).

NAFLD can progress from simple steastosis to liver inflammation and non-alcoholic steatohepatitis (NASH). NASH can further progress into cirrhosis. This accounts for 2-5% of NAFLD patients and has been shown to relate with type 2 diabetes mellitus (T2DM), obesity and age. NAFLD can also progress directly to fibrosis, at a slower rate (22).

NAFLD is typically diagnosed through imaging techniques (ultrasound, computed tomography, magnetic resonance imaging [MRI]), although NASH still requires a liver biopsy to confirm diagnosis, searching for specific characteristics of NASH such as hepatocyte ballooning and Mallory-Denk bodies. Due to the invasive nature of a liver biopsy, several efforts are being made to develop a method that can diagnose NASH without the need for a biopsy. In this regard, a study using mass spectrometry to identify specific serum metabolites, coupled with the presence of high levels of AST, fasting insulin and the PNPLA3 genotype has shown promising results (22).

There is still no pharmacological treatment for NAFLD, with most therapeutics strategies consisting in changes in lifestyle, i.e., a healthy diet − the Mediterranean diet has shown to be a good option − and some exercise and weight loss when needed, both for adults and children (22).

Nonetheless, some drugs have shown improvements in specific group of patients. For example, pioglitazone and vitamin E have been shown to reverse NASH and improve fibrosis in non-diabetic patients, with pioglitazone alone also proving effective in diabetic patients (22).

Novel putative therapies target the peroxisomal proliferative activated receptor (PPAR) family. A PPARα/δ agonist has proved to be beneficial in reversing NASH in patients with severe disease, as PPARα plays an active role in oxidative processes in the liver. Nonetheless, and despite all advancements in this area, it may be no single therapeutic strategy could benefit all NAFLD/NASH patients, because this disease can have different etiologies; and disease progression depends on various genetic and environmental factors (22).

5.3 Cirrhosis

Cirrhosis can be classified as end-stage liver disease, although it has recently been recognized as a dynamic process instead, resulting from any liver damage, such as viral hepatitis, alcohol damage, NAFLD, NASH, etc. The incidence of cirrhosis has been

increasing in developed countries as well as its morbidity and mortality rates, killing around 170 000 people in Europe every year. Unfortunately, there's still no known cure for this condition besides liver transplantation (23).

The mechanisms by which cirrhosis develops relate to liver inflammation and fibrogenesis - through activation of HSCs- , angiogenesis and hepatocyte damage. This will cause major histological alterations characterized by diffuse nodular regenerations with dense fibrotic septa, which lead to liver structure damage and collapse and cause important changes on hepatic vascular architecture. All of the referred alterations will lead to main consequence of cirrhosis: portal hypertension. Portal hypertension is measured by hepatic vein pressure gradient (HVPG) and is present when HVPG is higher than 5mmHg. It is caused by increased resistance to blood flow, as a result of all the histological alterations, and also by increased hepatic vascular tone. Then, an adaptive response is triggered in an attempt to reverse this process: splanchnic vasodilation. Although vasodilation should resolve the problem, being the direct opposite of increased vascular tone, it increases the blood flow into the portal venous system, worsening portal hypertension (23).

Cirrhosis usually remains asymptomatic until decompensation events occurs, which includes varices, ascites, encephalopathy, jaundice, splenomegaly, small and shrunken liver, among others, and can lead to organ failure and death in their acute forms. The diagnosis of cirrhosis relies on imaging methods (ultrasonography, MRI); although a tissue biopsy might be required for confirmation and also in cases of early cirrhosis, due to the risk of false negatives in imaging methods. During diagnosis, it is important to assess disease stage by screening for varices, particularly oesophageal varices. The disease can be divided into four stages, or even a fifth one: stage 1, with no oesophagel varices and a 1% annual mortality rate; stage 2, compensated liver function with oesophagel varices and a 3-4% annual mortality rate; stage 3, decompensated liver function with ascites and a 20% annual mortality rate and stage 4, decompensated liver function with gastrointestinal bleeding and a 57% annual mortality rate. A fifth stage might be considered in case of infections or renal failure and it presents a 67% annual mortality rate. After confirmation of diagnosis, it is important to follow up on patients to check on disease evolution and prevent the need for liver transplantation (23).

There is currently no targeted treatment for cirrhosis and the goal of existent therapeutic strategies is to avoid the need of organ transplantation. There are several non-

pharmacological options: lifestyle changes with weight loss when needed, along with adequate nutrition; alcohol abstinence is also recommended, as alcohol increases the risk of variceal bleeding; vaccination for hepatitis, influenza virus and pneumococcus are also recommended, as is smoking and cannabis cessation because it can worsen fibrosis (23).

Is also important to treat the underlying diseases, to avoid continuous progression, and to treat the consequences of cirrhosis such as controlling portal hypertension. Varices can be treated with non-selective β-blockers, or endoscopic band ligation.

In case of acute variceal bleeding, the patient should be quickly treat with an vasoactive drug and endoscopic therapy no later than 12h after bleeding. The patient should also receive a 5-day course of antibiotics to prevent further infection and even bleeding (23).

6. Hepatocellular Carcinoma

The two most common types of liver cancer are HCC and intrahepatic cholangiocarcinoma (iCCA) accounting for 95% and 4% of all liver neoplasms, respectively. The other 1% includes various other types of hepatic neoplasms, such as combined or mixed hepatocellular cholangiocarcinoma (cHCC-CC), fibrolamellar HCC (FLC) and pediatric hepatoblastoma (24, 25).

HCC is the second most common cause for cancer-related deaths worldwide and, unlike other cancer types, it is on the rise in many industrialized countries due to the increasing incidence of many HCC risk factors, including chronic HBV (33% of all cases), HCV (21% of all cases), alcoholic abuse (30% of all cases), metabolic problems (diabetes, obesity) and aflatoxin B1 intake. Tobacco smoking can also have an impact in HCC progression, and some recent studies show that adeno-associated virus 2 (AAV2) can also be a risk factor, especially in cirrhotic patients. (1, 26, 27)

HCC is commonly divided into five disease severity stages. Most cases of HCC are only discovered in an advanced stage, upon significant liver damage, leading to hepatic dysfunction. Therefore the development of prevention and screening programs all worldwide is critical. Primary prevention is done through HBV vaccination, antiviral treatment in case of chronic viral hepatitis and reducing risk factors (26, 27).

Different types of treatment have shown positive results in increasing the life time expectancy of patients, namely: surgical resection, liver transplantation, radiofrequency ablation, chemoembolization and sorafenib (26).

6.1 Epidemiology

HCC is the sixth most commonly diagnosed cancer, accounting approximately 850,000 cases per year, distributed heterogeneously across the globe due to different etiologies in different areas; different exposures to risk factors and different screening and treatments available locally (26-28).

The mortality pattern follow the etiological pattern, presenting higher age-standardized mortality rates (ASMRs) in Asia and sub-Saharan Africa, where HBV infections present higher age-standardized incidence rates (ASIRs) and less resources are available. Another factor that contributes to this heterogenous distribution is exposition to aflatoxin B1, that is much more common in Africa (1, 27). In North America, Japan and Europe, HCC shows much different patterns. Here, the main cause for HCC is chronic HCV infection, followed by metabolic factors, like obesity and diabetes. The median age of diagnosis is above 60 years, while in Asia and Africa it is usually between 30 and 60 years (26, 27).

HCC incidence rates have been rising in developed, high-income countries and it is predicted that they will keep increasing for the next 20 years (28).

6.2 Pathophysiology

HCC commonly develops from liver cirrhosis, which causes regenerating nodules, providing a microenvironment that benefits neoplastic lesions that initiate in normal hepatocytes (26, 27, 29).

Hepatocarcinogenesis is believed to be a complex, heterogenous process, with various important factors, including: tumor microenvironment, necroinflammation, oxidative stress, hypoxia and various other molecular processes (26, 29).

6.2.1 Natural history

As previously referred, HCC usually develops from liver cirrhosis. In particular, pre-cancerous low-grade dysplastic nodules (LGDNs) progress to high-grade dysplastic nodules (HGDNs) that develop into early-stage HCC (26).

Each HCC nodule (HGDN) accounts for about 40 genomic and driver cancer genes alterations, that accumulate, and are different from nodule to nodule, giving a unique pattern to each tumor (26).

One of the most common alterations is activation of the Telomere Reverse Transcriptase (TERT) promoter. TERT is responsible for the telomere stability and is not expressed in normal hepatocytes. Its activation occurs in ~44-65% of all HCC cases. Telomerase reactivation is the main cause for progression to HCC, promoting liver carcinogenesis. This reactivation is induced by two mutations in TERT promoter, at nucleotide positions -124 and -146, creating a new binding site leading to TERT mRNA expression. These specific mutations are found with great incidence in LGDNs (6%) but mostly in HGDNs (20%) and HCC (60%), proving that they are oncogenic (26).

Inflammation has an important role in hepatocarcinogenesis, contrary to other types of cancer. Hepatocyte inflammation creates an ideal environment for the development of genetic mutations through the release of different pro and anti-inflammatory cytokines and various transcription factors, which will initiate different signaling pathways, discussed further, that stimulate HCC development (29).

6.2.2 Molecular classes

The molecular mechanisms driving HCC development still need further investigation, giving that each HCC case has a different molecular pattern. Nonetheless, the accumulation of specifics genetic and epigenetic alterations during initiation, promotion and progression stages is already known (29, 30).

Some genetic studies have identified two main molecular classes of HCC, accounting about 50% of all cases, for HCC.

The first HCC molecular class is proliferative HCC, mostly found in HBV-related HCC and typically characterized by poor outcomes and aggressive tumors, leading to bad prognosis. As the name suggests, proliferative HCC relates with changes in signaling cascades involved in cell proliferation and include alterations in insulin-like growth factor (IGF), activation of RAS, activated protein kinase, among others. This HCC class also presents with high-level DNA amplifications of chromosome 11q13. This HCC molecular class is further characterized by molecular changes in both hepatocytes and progenitor cells (26, 30).

In turn, non-proliferative HCC involves changes only in hepatocyte cells, with a higher incidence in HCV and alcohol-related HCC; and with greater chance of better outcomes. This class is characterized by the activation of WNT signaling in ~25% of cases and by a disturbed immune response in remaining cases. Another specific characteristic of non-proliferative HCC is that the hepatocyte transcriptome remains physiologically normal (26, 30). Last but not least, some researches still include a third subclass of HCC, related to inflammation/interferon alterations (31).

6.2.2 Oncogenic drivers and tumor suppressors

Oncogenic drivers and tumor suppressors are the two main classes of genes on which mutations occur (31).

Tumor suppressor genes, like p53, are responsible for preventing tumor growth. They prevent cell proliferation, blocking division and inducing apoptosis (29).

Proto-oncogenes are also responsible for controlling cell proliferation and when they suffer mutations they transform into oncogenes, able to initiate carcinogenesis and becoming an oncogenic driver (Tables 3 - 5) (29).

Mutations typically occur in the promoter region of this genes, with TP53 and CTNNB1 being the most frequently mutated. Of note, TP53 and CTNNB1 mutations are mutually exclusive, which means that only one of them is found mutated in a particular tumor (31).

Table 3 Main oncogenic drivers involved in hepatocarcinogenesis (29, 31)

Oncogenic driver	Main function involved
TERT	Telomere stability
TP53	Genome integrity
CTNNB1	WNT signalling
ARIDA1A/ARID2	Chromatin remoddeling
NFE2L2	Oxidative stress
JAK1	JAK/STAT signalling

Table 4 Main proto-oncogenes involved in hepatocarcinogenesis (29)

Proto-oncogene	Main function involved
Ras family	Cell growth, differentiation and apoptosis
c-myc	Cell growth and differentiation
c-fos	Member of activating protein-1

Table 5 Main tumor-suppressor genes involved in hepatocarcinogenesis (29, 31)

Tumor-suppressor genes	Main function involved
pRB	Control of cell cycle progression
EGFR	Growth factor signalling
PTEN	AKT signalling
KRAS, NRAS	RAS/MAPK signalling

HCC development can also result from chromosomal alterations, usually amplifications. (26)

These can be divided into:

i) High level amplifications: in regions 11q13 (cyclin D1 [CCND1] and GF19) and 6p21 (VEGFA2); TERT focal amplification; homozygous deletion of CFKN2A; (31)

ii) Common amplifications: Myc (8q gain) and Met genes (focal gains 7q31). (31)

6.2.3 Signaling pathways

HCC signaling pathways and its many characteristics - such as how are they activated; which signaling pathway is activated in each case; among others - are not yet fully known.

The most common signaling pathways found altered in HCC are listed in Table 6.

Table 6 Signaling pathways in HCC development (29, 31, 32)

Pathway	Function	Mechanism	Prevalence
VEGF	Main role in angiogenesis	1) High level amplifications of VEGF-A and VEGFR-2 2) Ang2 and FGF signaling	7-10%
RAS/MAPK	Cell proliferation, growth, differentiation and survival	1) Upstream signaling of EGF, IGF, MET activation; 2) Epigenetic silencing of NORE1A and RASSF1A15	Half of early cases and almost all advanced ones
P13K/PTEN/AKT/mTOR	Cell proliferation, cell cycle and apoptosis	1) Activation by EGFR or IGFR (and other RTKs) 2) Inactivation by PTEN	40-50%
c-MET receptor+ligand HGF	Hepatocyte regeneration after liver injury	1) MET activation 2) Actived mutations or amplifications	1) 50% of advanced cases 2) 5%
IGFR	Proliferative and anti-apoptotic role, crucial to cellular and tissue regeneration	1) Allelic loss linked to IGFR2 2) Overexpression of IGF2 3) Deregulation of IGFBP2 and IGFBP3	20%
Wnt/β-Catenin	Hepatocarcinogenesis	1) B-catenin mutation 2) Frizzled receptors 3) E-cadherin inactivation	50%

6.3 Diagnosis, screening and prevention

Liver cancer, HCC being the most common, displayed a global incidence increase of 75% between 1990 and 2015, and is expected to keep on increasing for the next 20 years, despite being considered one of the most preventable cancers (1).

High HCC mortality mainly relates to its late diagnosis or the exitance of a previous liver disease that develops into HCC, which calls for the need of more and better screening and prevention programs worldwide.

6.3.1 Prevention

Primary prevention is defined as a group of preventive interventions applied, prior to any evidence of liver injury, and is considered the only realistic and sustainable approach to HCC prevention, diminishing the probabilities of liver damage and its progression to HCC. Secondary prevention is accomplished through surveillance of risk patients (27).

HCC usually arises from a chronic liver disease that can have different etiologies. To prevent HCC development, the correct strategy is to prevent these different causes.

The main HCC risk factors are HBV and HCV chronic infections, accounting for about 80% of all cases. HBV prevention can be done through vaccination, which is still not successfully achieved in low resource countries. In these countries, the risk mother-to-child infection during birth needs also to be taken into consideration. To prevent this, a study in Taiwan showed that pregnant women with high HBV viral load and who were given antiviral treatments during the third trimester of pregnancy showed reduced risks of mother-to-child HBV transmission. This is an important consideration to consider in less developed countries, where health care and vaccination programs are not enough to stop HBV transmission and reduce the HCC risk (1, 27).

As for HCV, and although a HCV vaccine is still not available, prevention measures include blood analysis before transfusion; and promoting the use of single-use needles and sterilization of surgical and dental instruments. These measures, coupled with intense and correct patient screening, may decrease HCV-related HCC incidence (27).

NAFLD is also a risk factor for HCC development and has become the leading cause of chronic liver disease, especially in industrialized countries. The risk of NAFLD

progression has been shown to be higher in the Hispanic population (not preventable), and in case of diabetes and obesity, both preventable (5, 27).

Alcohol is the second most common risk factor for HCC in developed countries, although it appears to be lower than that of with viral hepatitis. In case of alcoholic-related liver disease, the only preventive process is abstinence (27).

The last preventive risk factor for HCC development is exposure to aflatoxin B1 (AFB1) , which can be commonly found in cereal plantations in West African countries. AFB1 induces TP53 mutations and has a strong interaction with HBV, increasing the risk of HCC development upon simultaneous infection (27).

Last but not least, some drugs appear to decrease the risk for HCC and may be considered in high-risk patients. These include metformin, shown to decrease HCC development risk in both diabetic and non-diabetic patients, while statins have also proved to decrease risk in non-diabetic patients. Coffee- already recommended by European guidelines- and aspirin have also been shown to act as protective agents against HCC development (27).

6.3.2 Screening

HCC is usually curable when detected in an early stage. However, HCC is also usually asymptomatic in this stage, which makes it less probable to be promptly diagnosed (26).

The ideal technique for a correct and efficient surveillance of HCC is still not consensual. There is no doubt that ultrasound should be a part of it, but the use of biomarkers still remains controversial (26).

The guidelines for HCC surveillance recommend biannual surveillance tests for all high-risk patients, patients with chronic HBV infection and all cirrhosis patients, with or without other risk factors (27, 33).

Alpha-fetoprotein (AFP) serum levels represent the most common biomarker used to screen for HCC, even though it can present normal values in early stage HCC and their serum levels fluctuate in the presence of other liver diseases (33). Interestingly, a recent study showed that when ultrasonography is used simultaneously with AFP measurement, to screen patients with an interval of six-months - the surveillance time recommended by the American Association for the Study of Liver Diseases (AASLD)

and the European Association for the Study of the Liver (EASL) - a reduction of 37% in HCC-related was achieved (27, 33).

Other tumor biomarkers, like des-gamma-carboxyprothrombin (DCP), L3 fraction of AFP (AFP-L3) or glypican-3 (GPC3), are not recommended to be used in surveillance programs due to its low performance for early diagnosis. Nonetheless, DCP, AFP-L3 and AFP have been used in Japan for HCC surveillance, combined -with gender and age criteria- the GALAD score. This score has shown great results in early detection of HCC in both cirrhotic and non-cirrhotic CLD patients, with greater specificity to those with NASH. This is an advantage comparing to ultrasonography, which shows low accurate detection of early HCC stage in NASH patients, as these are often obese, leading to false results (27, 34, 35).

6.3.3 Diagnosis

For those patients who are enrolled in a surveillance program, HCC is more commonly detected in early stages, with identification of a new liver nodule. For patients who are not being surveilled, HCC is usually diagnosed in advanced stages, with large symptomatic tumors. Symptoms include weight loss, anorexia, abdominal discomfort and signs of liver dysfunction (26).

Diagnosis has to be confirmed in these cases, through either invasive or non-invasive techniques. Non-invasive techniques are favored, due to less complications, and include contrast imaging techniques, most commonly computed tomography (CT), MRI or contrast-enhanced ultrasound (CEUS). To confirm the diagnosis a new liver mass - with >1cm has to be detected, displaying specific features according to the used imaging technique (26, 27, 34, 36).

If both CT and MRI are not conclusive, patients are recommended to be followed in surveillance programs. However, if a liver lesion is found, in patients without cirrhosis or other risk factors, and non-invasive techniques cannot diagnose HCC, a liver biopsy is needed, even despite its possible disadvantages such as possible tumor seeding and bleeding and variation added by sampling and complications. If this tissue analysis is not conclusive, surveillance imaging is recommended every 3 to 6 months, with repeated biopsy if lesions aggravate (26, 37).

Serum biomarkers may be used in combination with radiological techniques. Recommended biomarkers include glypican 3, heat shock protein (HSP) 70, clathrin

heavy chain and glutamine synthetase. If two out of these four biomarkers are increased, there is a very high likelihood of HCC (38).

When nodules display <1cm in size, the recommendation is to follow-up the patient. In particular, EASL recommend ultrasound 3 times a year for the next immediate year, and AASLD recommends ultrasound every 3 to 6 months for two years (37).

6.3.4 Staging

Throughout the years, several HCC staging systems have been developed, including: the Hong Kong classification, the Cancer of the Liver Italian Program (CLIP), Japan Integrated Staging (JIS), International Hepato-Pancreato-Biliary Association (IHPBA), among others (39).

Nowadays, the only staging system validated, and accepted by both AASLD and EASL is the Barcelona Clinic Liver Cancer (BCLC) classification (39).

The BCLC classification, used since 1999, allows a wide analysis of the patient and tumor features, providing a correct decision to the treatment course. It includes criteria according to tumor burden, liver function and physical condition (performance status [PS]), and links these characteristics to a specific treatment, which is accounted has the best one for the disease stage (33, 34, 39).

The tumor characteristics include size, number of nodules, portal vein invasion and metastasis. The PS utilizes the Eastern Cooperative Oncology Group (ECOG) scale to analyze the general daily living ability of the patient. Finally, liver function is determined by the Child-Turcotte-Pugh (CTP) score (39).

BCLC classification includes 5 stages: very early (0); early (A); intermediate (B); advanced (C) and end-stage (D). Table 7 summarizes the main characteristics of each BCLC stage and indicated treatment approaches.

Of note, a disadvantage of the BCLC classification is that there is a high heterogeneity of patients in the intermediate and advanced stages. To address this problem, various sub-classifications have been proposed, but none of them was yet universally accepted (27, 39).

Another disadvantages of the BCLC classification is that it does not attribute a prognostic value to each stage, only a treatment approach (39).

Table 7 Summary of BCLC 5 stages main aspects (33, 39)

Stage	Main Aspects	Treatment approach
BCLC O Very early stage	Well-preserved liver function Asymptomatic Single tumor with <2cm in size CTP A; PS=0	Liver transplantation
BCLC A Early stage	Up to 3 nodules with ≤ 3cm Preserved liver function CTP A-B; PS=0	Liver resection Liver transplantation Radiofrequency Ablation
BCLC B Intermediate stage	Large multifocal tumors Without symptoms CTP A-B; PS=0	Transartherial embolization (TACE)
BCLC C Advanced stage	Extrahepatic spread and/or Portal vein invasion and/or Mild cancer-related symptoms CTP A-B; PS=1-2	Sorafenib
BCLC D End-stage	Poor liver function Not candidate for transplant Marked cancer-related symptoms Poor prognosis	Supportive care

6.4 Treatment

6.4.1 Liver resection

Liver resection is indicated as a treatment for HCC after consideration of very specific criteria, to prevent surgery risks and complications. It is usually recommended to noncirrhotic patients and cirrhotic patients with a single resectable HCC, with preserved liver function (Child-Pugh A), without macrovascular invasion and no portal

hypertension, which corresponds to the early stage of disease according to the BCLC classification. It can also be used in intermediate or advanced stages, for patients with preserved liver function in low resources countries, where better treatments are mostly unavailable (26, 27, 40).

Liver resection is considered a potentially curative treatment with low perioperative mortality and limited resection is preferred when possible.

Future liver remnant (FLR) has to be considered when planning this treatment, and this can be done through CT, predicting the risk of liver failure and complications after surgery. If FLR<20% or FLR<40% in cirrhotic patients, often when major resection is required, portal vein embolization (PVE) or lobar radioembolization can be used to increase growth of FLR, around 20-25%, diminishing the risk of post operation liver failure (26, 41).

The advance in new surgical techniques, such as laparoscopic surgery, have also transformed liver resection into a safer and lower complications option. For example, there is a reduction in blood loss, reducing blood transfusions, less postoperative liver failure and shorter hospital stay, decreasing the treatment costs. Laparoscopic techniques have also improved the major disadvantages of liver resection, this is, the recurrence of HCC (26, 40).

Recurrence of HCC after liver resection is common, accounting for about 70% at 5 years, and can occur as either metastatic or *de novo* HCC. In the presence of late recurrence, commonly 2 years after resection, it generally is *de novo* HCC, often in patients with advanced cirrhosis, males, of advanced age and with increased AST levels. Currently, there are no strategies to prevent HCC recurrence (26, 41, 42).

In case of recurrence after liver resection, the patient can still repeat resection, or if not possible, proceed to thermal ablation or liver transplantation (26).

6.4.2 Liver transplant

Liver transplantation was a big change in HCC therapeutic strategies because it has the advantage of removing the tumor and, if the disease etiology is also hepatic, the cause of the problem. This treatment is recommended for patients with early-stage HCC and when performed in this stage of the disease, the four-year survival rate is 75% with a recurrence rate of <10-15%, mostly extra-hepatic (lungs and bones). However, there is

two major issues regarding this strategy: the restrictive selection criteria for transplant and the limited organ availability (26, 40, 43).

The selection criteria for liver transplantation is called Milan criteria. It states a patient as candidate for transplant if one nodule ≤5cm or ≤3 lesions, none >3cm and absence of gross vascular invasion, metastases or lymph nodes. This criterion has existed for more than two decades and is accounted as the HCC features that allow for the better and longest outcomes for liver transplantation. As the Milan criteria excludes several patients from liver transplantation, and most of them are also not eligible for other curative treatments, various extensions to the Milan criteria have been suggested. The University of California San Francisco (UCSF) criteria suggests a single nodule up to 6,5cm or up to three lesions, none >4,5cm with the sum of diameters ≤8cm, with a patient fulfilling this criteria being able to undergo locoregional therapies to downstage the tumor until fulfilling the Milan criteria and therefore, being eligible for liver transplantation (27, 41, 43).

The liver transplant waiting list can go up to twelve months, which increases the risk of tumor progression, excluding the patient from Milan criteria, and with a dropout from the list in about 10-20% of the cases. To avoid this, bridging therapies are recommended by European guidelines. This depends on many disease factors, such as tumor location, size, number and hepatic function and can include therapies like liver resection and several locoregional therapies, of which radiofrequency ablation (RFA) and microwave ablation have been shown to be the most successful. These therapies can also be used to downstage tumor into Milan criteria (40, 43).

6.4.3 Locoregional therapies

6.4.3.1 Ablation

Percutaneous local ablation is the most common treatment for patients with early or intermediate stage HCC, with curative potential, and include RFA and microwave ablation (MWA), the two most used techniques, but also percutaneous ethanol injection (PEI) and cryoablation. All these different techniques involve injection of agents or energy, into the tumor, that will lead to necrosis of neoplastic cells. Tumor characteristics have to match criteria like tumor size (3-4cm), number (up until 3 tumors) and location (accessible with ultrasound guidance) (26, 27).

MWA and RFA techniques act through energy delivery. MWA suffers less interference of tissue factors, for example proximity to blood vessels, for which it shows better predictability and uniform ablation compared with RFA; as well as less heat sink effect, which leads to a better energy efficiency. This technique requires accurate needle placement to achieve good outcomes, since the tumor has to be caged in a microwave field. MWA is currently applied at two different frequencies: 915MHz, used for deeper tissue, with lower energy intensity and longer ablation time, used for smaller lesions; and 2.45MHz, with higher energy intensity, allowing a larger ablation, used for larger lesions (27, 44).

RFA is the first-line treatment to BCLC 0 and A patients that cannot undergo surgical resection. This course of treatment shows the best outcomes when used in hepatic tumors ≤5cm in size and with less than 3 nodules. Some trials were made to analyze if sorafenib in combination with RFA would be a better therapeutics option, but no statistic results were conclusive, for which RFA alone is still the recommended option. It presents a complete ablation rate of 99,4% with local recurrence of 3,2% at 10 years, although regional and extra-hepatic recurrence have a significant higher recurrence rate, about 74,8% at 5 years (44, 45).

PEI was the most used ablation technique until RFA and MWA were implemented. It is still commonly used in resource limited areas or in cases where the hepatic tumor is near large blood vessels or bile ducts, to avoid heating complications (27).

6.4.3.2 Transartherial Embolization

Transartherial embolization techniques include transartherial chemoembolization (TACE), transartherial radioembolization (TARE), also called selective internal radiation therapy (SIRT), and transartherial embolization (TAE) . Their mutual characteristic is the embolizing particles, which can differ from technique to technique, and that are released in the artery that feeds the tumor, embolizing it and leading to ischemic necrosis (27).

TACE is the main used technique, especially in bridging therapy, releasing one, or up to three, cytotoxic drugs - doxorubicin, epirubicin and cisplatin -, using an oily substance, often lipiodol, inside the tumor. TACE is also commonly used in nonresectable HCC to improve overall survival (OS) and quality of life, although TACE efficacy depends on tumor extent and liver dysfunction degree, with a OS rate of 23%

in Child-Pugh class A patients and OS rate of 13% in Child-Pugh class B patients. TACE therapeutic scheme is usually designed accordingly with the patient needs, with CT or MRI evaluation every 2 months, and more therapy sessions if needed. Patients with portal vein thrombosis are not suitable candidates for TACE (26, 27, 40, 45).

Some recent trials comparing TACE with TAE, which relies only on mechanic embolization of the arteria, shown no different between their outcomes, in OS, treatment response or adverse events, with TAE reducing treatment toxicity. These evidences need confirmation, for which TACE remains the recommended course of treatment (27, 45).

Contrary to TACE, TARE can be used in patients with portal vein thrombosis, tumor invasion, downstaging nonresectable HCC or radiation lobectomy. TARE functions through microspheres loaded with β-emitting yttrium-90 isotope, which are released into the artery that feeds the tumor, just like in TACE (27).

6.4.4 Sorafenib

Sorafenib was the first approved molecular therapy for HCC. It's a small multikinase inhibitor, and although it's mechanism of action is not yet fully understood, it is known that it targets different kinases such as VEGFR and platelet-derived growth factor receptor (PDGFR), blocking their mechanisms. It has few, manageable side effects, for example diarrhea and skin reactions (26).

This is the only drug that have shown survival advantages, so there is an urgent need for new therapeutic options (26).

7. New treatments

7.1 Oncolytic virotherapy

The first attempts to use oncolytic virotherapy (OVT) to treat cancer dates from the 1950's. In the last years, given all scientific and technological advances, OVT approaches have been rising, with some have already showing excellent results and having been approved for use in the clinics (46, 47).

An oncolytic virus (OV) does not only performs a transportation function, and is also considered an active drug. An OV is a virus that can rapidly replicate and kill cancer cells, through lysis or apoptosis, without damaging healthy cells. It can be genetically engineered or occur naturally, the later observed in cases of tumor regression after systemic viral infection. In addition, OVT can also stimulate the immune system to act against the tumor (47, 48).

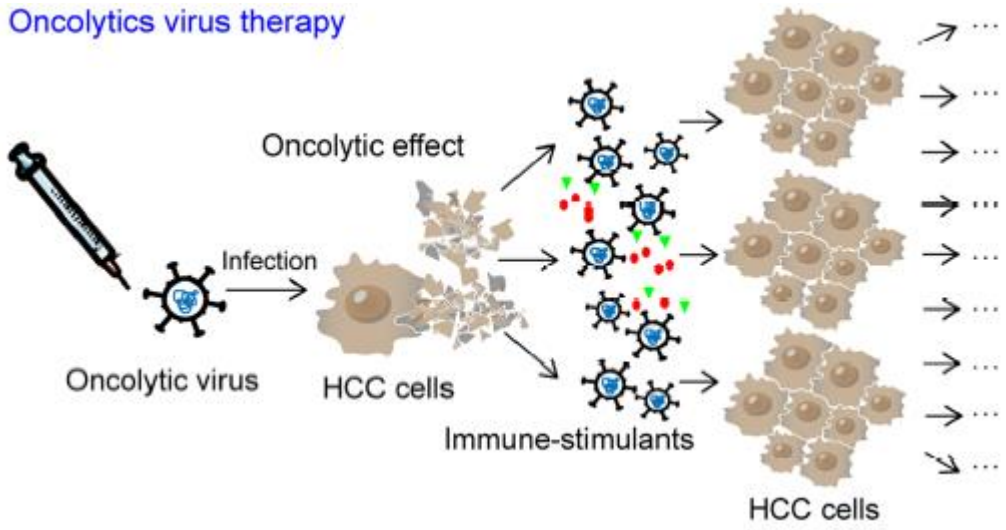

Figure 2 Mechanism of action of oncolytic virus therapy. Oncolytic viruses replicate only in tumor cells, spreading the virus in tumor tissue and not damaging healthy cells.

Figure from (49).

Not every virus can become an OV. Specific aspects that are intrinsic to all OV, include selective targeting to damaged cells, rapid replication to avoid being neutralized by the immune system, not being on the list of population immunized virus and being safe. These features do not have to be naturally present, as virus can be genetically modified to be transformed into an OV (47).

There are several HSV oncolytic vectors on trials and one of them, Oncovex^{GM-CSF}, has made its way up to a phase III clinical trial for metastatic melanoma (46). To evaluate the possibility of OVT in HCC, researchers from the Breast Cancer Center in China developed a trial with a G47Δ oncolytic vector in HCC cell lines.

These researchers used a Herpes Simplex Virus (HSV) oncolytic vector. The oncolytic HSV G47Δ was genetically modified, as described in Table 8. The oncolytic HSVs exhibited good safety and specificity profiles, acting through an oncolysis mechanism.

They do not interfere with other therapies, and so they can be used as an adjuvant treatment (46).

Table 8 Oncolytic HSV G47Δ mutations (46)

Gene	Normal Function	Mutation	Consequences	OVT advantages
γ34.5	Shut-off of protein synthesis	Deletion	Limiting virus replication	Targeting, exclusively, cells with innate immune responses (cancer cells)
UL39	Encoding ICP6 (key enzyme to DNA synthesis)	lacZ insertion	ICP6 inactivation; Higher sensitivity to acyclovir and ganciclovir;	Virus DNA replication blocked in normal cells
ICP47	Inhibiting viral particles to TAP proteins	Deletion of α47	1) Placing US11 gene under α47 promoter control; 2) Increasing MHC class I presentation	1) Amplification of γ34.5 mutant growth; 2) Enhance of antitumor immune responses.

For evaluation of the oncolytic HSV G47Δ, several cell lines were used HepG2, Hep3B, SMCC-7721, BEL-7404 and BEL-7405 HCC human cell lines and HL-7702 human hepatic immortalized cells. The first three cell lines suffered 95-100% death 5 days after infection, the BEL-7404 lineage suffered 70-90% cell death 5 days after infection and BEL-7405 were the less sensitive ones, with 29.9-57.7% cell death 5 days after infection. There were also some *in vivo* studies, using mice with BEL-7404 and SMCC-7721 tumors, which demonstrated complete tumor regression in, respectively, 4 of 6 mice and 5 of 6 mice (46).

These results suggest that G47Δ, with no signs of toxicity until date, can effectively kill a series of HCC human cell lines, embodying a promising therapeutic strategic, especially for advanced stage HCC, where surgery is not a viable option. In addition, it

could also be used has a preventive treatment for patients with advanced liver disease (46).

7.2 Immunotherapy

The field of immune-based therapies is rising and has already demonstrated promising results in various malignancies (50).

The development of immunotherapies requires the identification of tumor antigens, that can be recognized by the immune system. There are three types of tumor antigens: tumor-specific antigens (present only in cancer cells); tumor-associated antigens (TAA) (majorly in cancer cells but low levels can be detected in normal cells); and cancer/testis antigens (cancer and reproductive cells). Currently, the number of known HCC tumor-specific antigens is low, with most studies using AFP (51).

HCC is characterized by having a tumor micro-environment (TME), with an important role in tumor growth promotion and immune response, allowing for immune tolerance of cancer cells, through adaptive immune responses. The TME can be targeted through inhibition of two immune checkpoints: Cytotoxic T Lymphocyte-associated protein 4 receptor (CTLA-4) and Programmed Cell Death protein.1 receptor (PD-1). Most trials using immune-based therapies for HCC are based on immune checkpoint inhibitors (50, 51).

Cell based therapies, cytokines and vaccines are also in development, although to a smaller extent (51). In fact, the only immune-based therapy drug approved – nivolumab – is an immune checkpoint inhibitors. In particular, Nivolumab is a PD-1 inhibitor, second-line approved drug, in cases of intolerance or progression with sorafenib, for which the majority target of patients display advanced diseased, with extrahepatic metastases. In the CheckMate 040 trial, nivolumab showed complete response (CR) in 3 patients and partial response (PR) on 30 patients, among 212 patients (51).

There are two other immune checkpoint inhibitors drugs in development: tremelimunab and pembrolizumab. The first one is a CTLA-4 inhibitor in a phase II trial, for patients with chronic HCV infection. Results showed PR in 18% of the patients and stable disease (SD) in 59%. The second, pembrolizumab, is a PD-1 inhibitor, like nivolumab, showing similar response rates in a phase II trial. Nonetheless, pembrolizumab failed to meet the primary end points in a phase III trial, for which further studies are needed (51).

Cell based therapy, specifically Adoptive Cell Transfer (ACT) therapy is considered the most personalized form of immunotherapy. It consists on extracting, modifying and/or amplifying immune cells from a patient, and the transferring them back to the same patient. There are two main types of immune cells to this treatment: tumor infiltrating lymphocytes (TILs) and cytokine-induced killer (CIK) cells (51, 52).

TILs are antitumor effector cells which may be rare in HCC but have a specific role in tumor recurrence and patient prognosis, making them extremely relevant as a treatment option. ACT treatment basically consists of TILs infusions, which have been demonstrated to reduce recurrence rates and increase five-year survival rates by over 30% in patients who suffered liver resection. However, TILs are difficult to isolate and amplify and few patients can tolerate lymphocyte deletion, a process needed before TIL infusion (52).

CIK cells are expanded from the peripheral blood mononuclear cells (PBMC) with added anti-CD3 antibodies and recombinant human IL2, which improve the CIK cytolytic activity (Figure 2). They are considered to possess very potent antitumor properties, being able to recognize and kill tumor cells directly. Like TILs, they have been used in combination with liver resection and have shown improved OS (51, 52).

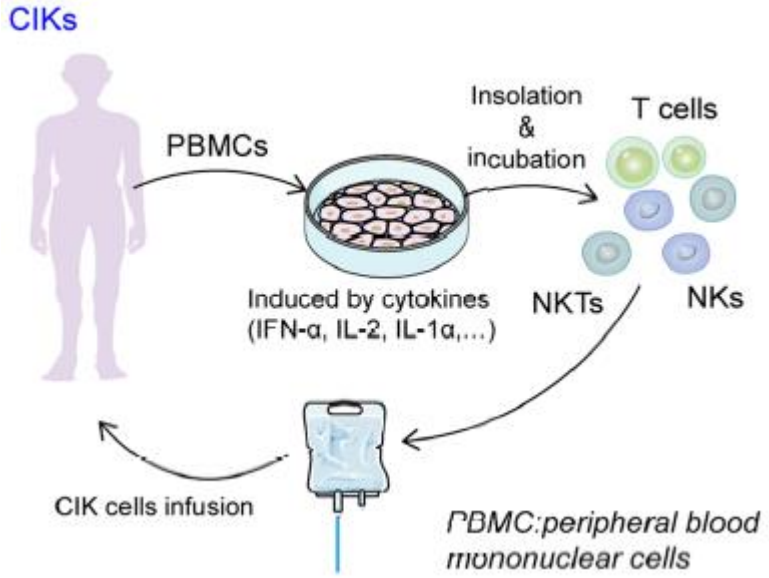

Figure 3 Representation of adoptive cell therapy using cytokine-induced killer cells: Cytokine-induced killer cells are obtained from peripheral blood mononuclear cells (PBMCs), expanded in the presence of cytokines.

Figure from (49).

7.3 Novel pharmacotherapies

Currently, the only commonly used drug to treat HCC is sorafenib, although there are recently approved new drugs, namely Lenvatinib (first-line); cabozantinib and regorafenib (as second-line), recommended by EASL since 2018 (Figure 2) (53).

Lenvatinib was the second drug discovered for first-line HCC treatment, almost 10 years after sorafenib, showing non-inferiority results in Lenvatinib versus sorafenib trials. Lenvatinib is a rapidly absorbed oral multikinase inhibitor of VEGR, FGRs, RET, KIT and PDGFRα, having a wide action spectrum and a special feature that differentiates it from other multikinase inhibitors: it potently inhibits FGFR-1. In phase III trials, Lenvatinib led to an OS of 13.6 months versus 12.3 months with sorafenib, evidencing Lenvatinib benefits for patients with advanced HCC (36, 54).

Regorafenib has a similar mechanism to sorafenib, inhibiting multiple protein kinases responsible for angiogenesis (VEGFR), oncogenesis (KIT, RET, BRAF); and also proteins of the MAPK, apoptosis and autophagy signaling pathways, that is, the tumor microenvironment. Trials with regorafenib showed improved survival in patients resistant to sorafenib, for which. The EASL guidelines recommend regorafenib for HCC patients previously treated with sorafenib (36, 55, 56).

Finally, cabozantinib, majorly targets MET, VEFDR2, ACL and RET, but also KIT and FLT3. The trial for HCC treatment using cabozantinib showed an OS of 10.2 months versus 8.0 months in the placebo (55, 57).

Ramucirumab is a monoclonal antibody targeting VEGR-2 which failed to meet the primary endpoint in phase III trial for HCC. Very recently, in another phase III trial on a specific HCC sub-population - patients with high baseline AFP - ramucirumab led to improved OS when comparing to placebo, joining cabozantinib and regorafenib as a second-line treatment for advanced stage HCC (36).

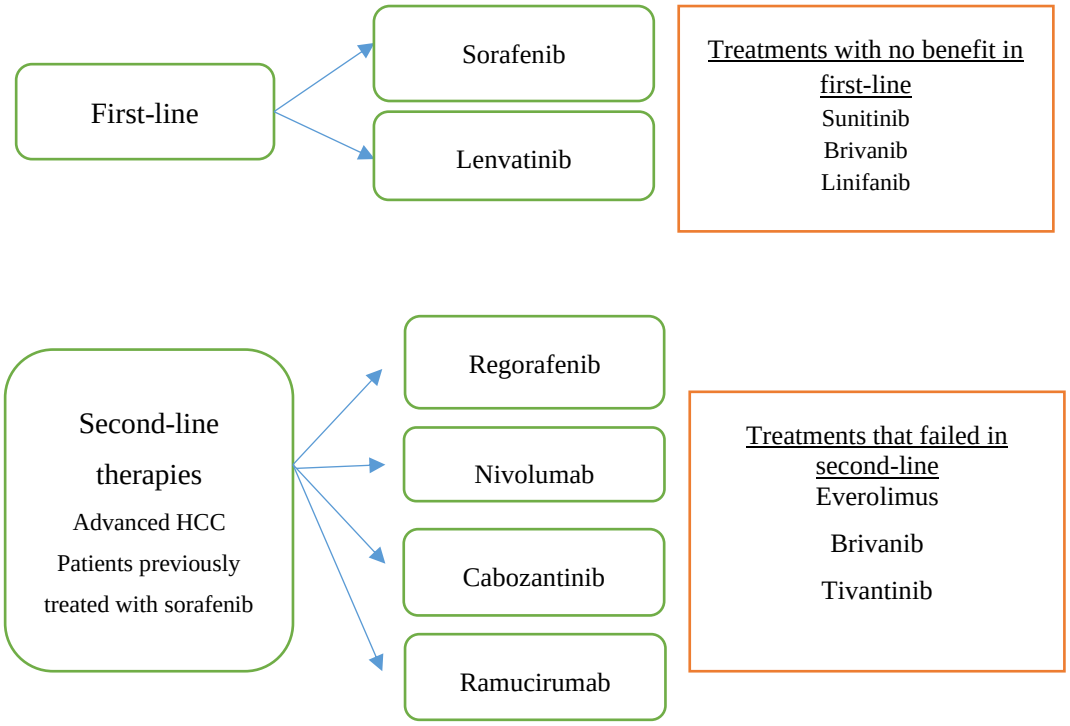

Figure 4 Pharmacologic therapies for HCC approved by EASL.
Hepatocellular carcinoma is considered one of the most chemo-resistant tumor types, which makes pharmacologic therapy crucial to treatment of advanced stage disease.

Figure based on (36).

7.4 Proton Beam Therapy

It was not until recently that radiotherapy (RT) was included in the array of treatment options available for HCC, due to the risk of both cancer and healthy cells being exposed to radiation (45).

Advances in Proton Beam Therapy (PBT) have brought innovation to RT treatments or HCC, as it shows good results with minimal risks of the remaining liver and healthy organs being exposed to radiation. This is because while X-ray releases some energy on the beam path, depositing and affecting the normal function of healthy tissues, the proton beams have a finite range of deposition, losing less energy along the way, reducing the risk of healthy tissues damage. This applies especially to patients with advanced cirrhosis and poor liver function, which are not candidates to RT due to this risk, but to whom PBT can be applied (58). PBT is included in RT options for HCC and presents itself as a local alternative to embolization and ablation techniques for patients

that are not suitable for surgical approach, being only currently offered TACE and sorafenib (58).

PBT can act by two different mechanisms: directly through collisions with DNA molecules, which cause damage and cytotoxicity; or indirectly through radical free oxygen species production that cause DNA damage (58).

Although PBT indications are still being studied, several trials that showed promising results in patients with portal vein tumor thrombosis, for which the current treatment recommendation is only sorafenib (2- and 5-years OS rates of 88% and 58%); patients with large HCCs (>10cm) with 2-year OS rate of 36%; patients with poor liver function (2-years OS rate of 42%); and patients with larger liver metastases. All of these applications require further studies (58, 59). PBT is also a local alternative to embolization and ablation techniques for patients that are not suitable for surgical approach, being only currently offered TACE and sorafenib (58).

Although a promising technique to improve OS and quality of life in HCC patients with few therapeutic options, PBT has also some disadvantages. Even with a finite range of energy deposition, the exact PBT range is still not defined, ultimately targeting organs surrounding the liver, including the GI tract, biliary tract and chest wall. These organs should be protected from radiation, for instance using surgical tissue expanders. Further there are range alterations according to the material density, which can be changed through by organ motion. To overcome this issue, there is the need for imaging guidance, to ensure that radiation only affects the minimal tissue needed. (58, 59)

8. Future Perspectives and Conclusion

Although HBV vaccination have proved effective in decreasing the HCC burden, especially in low resource areas, HCC still impacts global health due other etiologies. In the last years, obesity, diabetes and other metabolic disorders have been increasing in industrialized countries, leading to increased incidence of HCC.

At the same time, novel therapeutic modalities are being developed. Immunotherapy is already used for several cancer types and shows promising results in HCC; pharmacotherapy options are increasing as well as its accuracy. Nonetheless, because HCC tumors have a very specific microenvironment and can exhibit over 40 different

mutations, differing from patient to patient, as well as different etiologies, finding a therapeutic option that can be applied globally is almost an impossible task.

Although efforts have to be made to continue research in new treatment options, the main course for decreasing the HCC burden remains through screening programs, mainly in high risk patients, with previous liver disease, or patients with risk behaviors. A worldwide screening program, although with moderate financial costs, would be balanced by the decrease of health care expenses with cirrhotic and HCC patients, especially those in advanced stages.

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