ANNALS of Hepatology

CONCISE REVIEW

April-June, Vol. 9 No.2, 2010: 120-132

Prevention of hepatocellular carcinoma

Michael C. Kew*

* Department of Medicine, University of Cape Town, Cape Town, and
Department of Medicine, University of the Witwatersrand, Johannesburg, South Africa

ABSTRACT

Because of its frequency and grave prognosis, preventing hepatocellular carcinoma is an urgent priority. Prevention should be possible because environmental carcinogens-chronic hepatitis B and C virus infections, dietary exposure to aflatoxins, and iron overload-cause the great majority of these tumors. Chronic hepatitis B virus infection accounts for 55% of global hepatocellular carcinomas and 80% of those in the high-incidence Asia Pacific and sub-Saharan African regions. In these regions the infection that becomes chronic is predominantly acquired very early in life. A safe and effective vaccine against this virus is available and its universal inclusion in the immunization of infants has already resulted in a marked reduction of chronic infection and a 70% decrease in the occurrence of hepatocellular carcinoma in those immunized. Chronic hepatitis C virus infection is the major cause of hepatocellular carcinoma in industrialized countries. The infection is mainly acquired in adulthood and, until a vaccine becomes available, prevention will consist mainly of identifying, counselling, and treating chronically infected individuals, preventing spread of the virus by the use of safe injection practices (particularly in intravenous drug abusers), and screening all donated blood for the presence of the virus. 4.5 billion of the world's population are exposed to dietary aflatoxins. Prevention involves treating susceptible crops to prevent fungal contamination, and handling the foodstuffs in such a way as to prevent contamination during storage. Iron overload in hereditary hemochromatosis can be prevented by repeated venesection and in African dietary iron overload by fermenting the home-brewed beer in iron-free containers.

Key words. Hepatitis B virus. Hepatitis B virus vaccine. Hepatitis C virus. Cirrhosis. Aflatoxin. Iron overload.

THE NEED TO PREVENT HEPATOCELLULAR CARCINOMA

Among the reasons for the belief that HCC is one of the major cancers in the world today are two that are particularly germane when considering the need to prevent the tumor. The first is the high incidence of HCC and the second its extremely poor prognosis.

HCC is the sixth most common global cancer,[1,2] with approximately 630,000 new cases occurring each year, accounting for about 5.7% of all new human cancers.[3] Its incidence differs considerably in different geographical regions, with more than 80% of cases occurring in resource-poor countries in the Asia-Pacific region and sub-Saharan Africa.[1-3] In addition, the incidence of the tumor is increasing in a number of countries in which it previously had a low or an intermediate occurrence rate. HCC ranks third in annual global cancer mortality rates, and has the shortest survival times of any cancer in both males and females.[1-4] The prognosis is especially grave in Chinese and Black African patients, in whom the annual fatality ratio is 0.97 and the tumor is responsible for as many as two-thirds of cancer deaths.[4] Although some improvement in the results of treating HCC have recently been achieved in resource-rich countries, this has had little impact on the overall mortality rate of the tumor.

As a consequence of the often rapid growth of HCC, especially in Black African and Chinese patients, and the absence of symptoms during the early stages of the disease, the tumor is usually at an advanced stage when the patients are first seen.

Correspondence and reprint request: Professor M.C. Kew, K Floor.
Old Main Hospital Building, Groote Schuur Hospital, Main Road, Observatory 7935, Cape Town, South Africa
Telephone: 27 021 782 2949 Fax: 27 021 448 6815
E-mail: Michael.Kew@uct.ac.za

Manuscript received: March 05, 2010.
Manuscript accepted: March 17, 2010.

So prevalent is HCC, especially in many populous resource-poor countries, so poor are the results of treatment when the tumor is symptomatic, and so grave is the prognosis, that prevention of HCC is an urgent priority. Because the etiology of HCC is known in more than 90% of the patients and the great majority of these causes are potentionally preventable, prevention of almost all of these tumors should theoretically be possible.

CAUSES OF HEPATOCELLULAR CARCINOMA

The commonest and geographically most widely distributed of the recognised causes of HCC are chronic HBV and HCV infections and cirrhosis, whatever its cause. Other risk factors are important in certain geographical regions only: dietary exposure to the fungal toxin, aflatoxin, in parts of sub-Saharan Africa, the People's Republic of China, and Taiwan; dietary iron overload in parts of sub-Saharan Africa; NASH, mainly in resource-rich industrialized countries; ditch, pond, or river water contaminated by blue-green algae that produce tumor-promoting microcystins in parts of the People's Republic of China; and membranous obstruction of the inferior vena cava in Nepal, South Africa, Japan, the People's Republic of China, and Korea. Rare causes include a number of inherited metabolic diseases, the most common of which is HH.

The prevention of only the more common of the causes of HCC will be considered in this review.

Chronic hepatitis B virus infection

Approximately 45% of the world's population live in regions endemic for HBV infection.[5] More than 2 billion people worldwide are estimated to have been exposed to HBV, of which some 360 million (approximately 6% of the global population) are chronically infected with the virus.[4,6] Chronic HBV infection is the predominant cause of HCC worldwide, being responsible for approximately 55% of the tumor throughout the world and about 80% of the tumor in the Asia-Pacific region and sub-Saharan Africa. Of those individuals chronically infected with the virus, one-quarter or more will develop HCC.[1,2,4] Each year between 500,000 and 700,000 individuals chronically infected with HBV die from HCC, cirrhosis, or both diseases.[5]

In eastern and south-eastern Asia, Melanesia, and sub-Saharan Africa, with HBV carrier rates as high as 15% and a very high incidence of HCC, the infection is predominantly acquired in infancy or early childhood. The main route of infection in Chinese populations is perinatal transmission of the virus from highly infectious HBV e antigen-positive carrier mothers to their newborn babies. Fewer infections occur a little later as a result of horizontal transmission of the virus.[7] In contrast, relatively few infections in Black African children are the result of perinatal transmission, most infections being acquired a little later by horizontal spread of the virus.[8] Young siblings or playmates, recently infected and hence highly infectious, are the major source of the horizontal transmission, although other family members, especially the mother, also play an important role. HBV infections acquired during the first year of life have a 90% chance of becoming chronic and those acquired between one and five years a 30-50% chance.[9] This contrasts with a risk of less than 5% when HBV infection is acquired in adulthood. These early onset carriers are at very high risk of HCC development later in life, with lifetime relative risks as high as 100.[10] The risk of malignant transformation is even greater when cirrhosis is present.[11] Because the majority of the older population in these countries are already immune to HBV infection, and HBV infections acquired at these ages seldom become chronic,[9] viral transmission later in life is uncommon.

Chronic hepatitis C virus infection

Chronic HCV infection is another important global cause of HCC, and is a major risk factor for the tumor in many industrialized countries. Some 170 million people worldwide are currently estimated to be chronically infected with this virus.[4,12] The infection is almost always acquired in adulthood, mainly as a result of the illicit use of injectable drugs and sexual transmission. Eighty percent or more of individuals acutely infected with HCV become chronic carriers of the virus and about 60% develop chronic hepatitis. Of the latter, approximately 20% progress to cirrhosis over a period of 20 to 25 years, and a portion of these develop HCC[12,13] The annual risk of tumor formation in those chronically infected ranges from 8-9% in Japan to 1.9% in the United States, the risk being at least four-times greater in those with cirrhosis.[12,13] Other risk factors are a high viral load, increasing age, male gender, and more severe degrees of hepatic fibrosis.[13]

During recent decades, the incidence of HCV-related HCC has increased two- to three-fold in Japan and Egypt and to a slightly lesser extent in the Uni-

ted States, Canada, the United Kingdom, some western European countries, and Australia.[4] HCV is the major cause of HCC in Japan (which has a high incidence of HCC), Spain, Italy and Egypt (with intermediate incidences of the tumor), and a number of industrialized countries with a low incidence of the tumor.[4]

Cirrhosis

All etiological forms of cirrhosis may be complicated by the development of HCC, although not with equal likelihood.[14] The main causes of cirrhosis complicated by malignant transformation are chronic HCV infection, chronic HBV infection, habitual alcohol abuse, and, to a lesser extent, NASH. Although chronic HCV infection and alcohol abuse may occur together in industrialized countries, the exact nature of their interaction in hepatocarcinogenesis remains to be determined.[15] Apart from the etiology of the cirrhosis, the major factors predisposing to malignant transformation in cirrhotic patients are increasing age, duration of cirrhosis, and male sex.[14,15] Reactive oxygen and nitrogen species generated by the chronic necroinflammatory hepatic disease (chronic hepatitis and cirrhosis) are mutagenic and carcinogenic,[16] with the increased hepatocyte turnover rate acting as a potent tumour promoter.

Aflatoxins

Aflatoxins are structurally related difuranocoumarin derivatives produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*. These fungi are ubiquitous, but because humidity and moisture content of plants are important factors in determining growth and toxin production by the moulds, contamination of crops occurs particularly in tropical and sub-tropical countries with warm, humid climates. Certain staple foodstuffs, especially maize, ground nuts, and fermented soy beans, are particularly prone to contamination,[17,18] especially in subsistence farming communities. Contamination occurs not only during growth of the crops, but also as a result of their improper storage.

Of the aflatoxins, AFB_1 is most often found in contaminated human foodstuffs, and is the most potent hepatocarcinogen in both humans and experimental animals.[17,18] The hepatocarcinogenic effects of AFB_1 and HBV are synergistic, with multiplicative relative risks for HCC development.[19]

The liver is the primary site for biotransformation of AFB_1. Although the parent molecule is innocuous, it is converted by members of the cytochrome P450 super-family in the phase I pathway into electrophilic intermediates.[17,18] The *exo*- and *endo*-epoxides formed are detoxified by a number of phase II pathways, principally by glutathione-S-transferase-mediated conjugation.16 If the quantity of AFB_1 ingested in the diet exceeds the capacity of the phase II pathways to detoxify the epoxides formed or if, for any reason, the activity of these pathways is decreased (for example, by polymorphisms of the glutathione-S-transferase gene), the highly reactive accumulated exo-epoxide binds with high affinity to guanine bases in cellular DNA to form the AFB_1-N^7-gua adduct.[17] AFB_1-N7-Gua can be converted to the more persistent AFB_1-FABY adduct. These adducts give rise to guanine to thymine transversions in cellular DNA. AFB_1-induced promutagenic changes can result in the activation of proto-oncogenes and inactivation or loss of tumor suppressor genes, with malignant transformation of hepatocytes as the final outcome.

Iron storage diseases

Although essential for the growth of cells, in excessive amounts iron is toxic. The liver is especially subject to this toxic effect because it is the major site of iron storage. Iron storage disease occurs in two main forms-HH and dietary iron overload in the Black African. HH is an autosomal recessive disorder that results in increased absorption of elemental iron from a diet containing normal amounts of the metal. Portal fibrosis, cirrhosis, and HCC are frequent complications of the resulting iron accumulation.[20] HCC is responsible for as many as 45% of deaths in patients with HH, with a relative risk of greater than 200: the longer the patient survives the greater is the risk.[20] A substitution of tyrosine for cysteine at amino acid 282 of the α3 loop (C282Y) of the HFE protein is the mutation most often responsible for HH.

Cirrhosis was thought to be an essential precursor of malignant transformation of hepatocytes in HH. However, in recent years more than 30 patients with the disease but without cirrhosis have been reported to develop HCC, suggesting the possibility that excess hepatic iron may be directly hepatocarcinogenic, in addition to causing HCC indirectly through the supervention of cirrhosis. Further evidence is provided by the observation that the risk of HCC in patients with cirrhosis complicating HH is greater than that in patients with cirrhosis attributable to other causes.[21]

Increased levels of hepatic iron, on a par with those present in HH, are found in Black Africans with dietary iron overload.[22] This condition occurs in many rural areas of sub-Saharan Africa, where as many as 15% of the men may be iron-overloaded.[23] It results from the consumption of large quantities of traditional alcoholic beverages with a high iron content as a result of being brewed in iron drums or pots. During the process of fermentation, the pH of the ferment drops to a very low level, leaching iron from the container into the contents. Large quantities of the beverage are consumed because it has a low alcohol content.

Portal fibrosis and cirrhosis complicate dietary iron overload in significantly fewer patients than occurs in HH.[22,24] Nevertheless, HCC develops, with a relative risk of 10.6 (95% confidence limits 1.5-76.8) and a population attributable risk of 29.[25] Further support for a direct carcinogenic effect of excess hepatic iron is provided by the formation of iron-free preneoplastic foci and HCC, in the absence of cirrhosis or portal fibrosis, in an animal model of dietary iron overload[26] Malignant transformation in this model appears to be induced as a result of the generation of reactive oxygen species by the increased hepatic iron.[26]

Microcystins

In most rural regions of the People's Republic of China with a high incidence of HCC, the population drinks primarily pond or ditch water. Drinking water from these sources or, to a lesser extent, river water rather than deep-well water, is a risk factor for HCC in some of these regions, relative risks of 1.9 (95% confidence interval 1.01-4.74) and 2.9 (95% confidence interval 2.59-3.27) being recorded in Haimen and Fusui, respectively.[27] Tumor- promoting microcystins derived from blue-green algae have been identified in pond and ditch water in these high incidence regions of HCC, and differences in the microcystin content of the drinking water have been recorded between HCC patients and controls.[28]

PREVENTION OF HEPATOCELLULAR CARCINOMA

Attempts at preventing HCC are of relatively recent origin, but there is every prospect that prevention of most cases of this common and devastating tumor will be possible in the not-too-distant future. For the immediate future the emphasis should be on practical and economical interventions in countries with high incidences of HCC, especially resource-poor countries. Because of the different patterns of the causes of the tumor in different geographical regions, strategies for prevention will need to be tailored for each such region.

Primary prevention

Primary prevention, defined as preventing the etiological agent from initiating the carcinogenic process, is, at least theoretically, the most effective form of cancer prevention. It lends itself particularly well to intervention in viral, chemical, and physical causes of cancer. Given that oncogenic hepatitis viruses contribute to the development of more than 80% of global HCC, prevention of these chronic infections alone would have an immense impact on the global occurrence of the tumor. Primary prevention could most effectively be accomplished by universal immunization against the viruses. This approach became possible when an effective and safe vaccine against HBV became available in the 1970s.

- ***Immunization against hepatitis B virus infection:*** Since 1991 the WHO has recommended that HBV vaccine be included in the routine infant immunization program in all countries.[29] The vaccine is currently incorporated into the EPI in 168 countries, with a global coverage of infants of 60% in 2006. In those countries in which HBV infection is endemic and universal infant immunization is in place, 80-90% of babies are now receiving a full course of the vaccine. This accomplishment has already resulted in a decrease from 90% to 15% in the percentage of chronically infected babies born to highly infectious carrier mothers, and a 10-fold or more decrease in the rate of chronic HBV carriage in the age groups that have been immunized.[30,31] Because of the usually long interval between the initial infection with HBV and the development of HCC, it will take 25-50 years for a significant decrease in incidence of HBV-induced HCC in adults to be realised in these countries. Nevertheless, in Taiwan, where immunization of babies against HBV began in 1984 and universal coverage was achieved by 1986, coverage of all preschool children by 1987, and extension to older school children, teenagers, and adults by 1990, the prevalence of HCC among recipients of the vaccine has decreased by 70% in comparison with those in the non-vaccinated age groups.[30] In early reports, the response rate was higher in boys than

girls,[31] but in later analyses based on larger numbers of children this difference was not evident.[32] With the passage of time the decrease in incidence of HCC in those immunized became evident in adolescents and recently in young adults. These findings augur well for the eventual elimination of HBV infection and HBV-induced HCC in the Asia-Pacific region and have shown the way for other countries and regions to follow. In the few "break-through' cases of HCC still seen, failure to complete the full course of immunization was responsible.[33]

In sub-Saharan Africa, for a number of reasons but principally because of financial constraints, competing health care priorities (HIV/AIDS, malaria, tuberculosis, measles, and diarrheal illnesses) and poor delivery services not able to access large parts of the countries, only 10% of babies were until quite recently being immunized against the virus. However, with the provision of financial backing from the Global Alliance for Vaccines and Immunization, the Vaccine Fund, and other governmental and non-governmental sources the dismal response in sub-Saharan Africa is now changing for the better.[34,35] Approximately 60% of Black African infants are currently being immunized.

The encouraging results achieved in the Far East give promise that the universal incorporation of HBV vaccine into the EPI in countries in which HBV infection is endemic will in the future prevent hundreds of thousands of deaths each year from HCC and cirrhosis,[35] and that with global immunization HBV-induced HCC could ultimately be completely prevented.

Because of the early acquisition in highly endemic regions of HBV infection that becomes chronic, immunization should be performed at or shortly after birth. In populations in which perinatal transmission from highly infectious HBV e antigen positive mothers to their babies is the predominant mode of infection, the highest level of protection against the virus is achieved when the first dose of the vaccine is given as soon after birth as possible, together with HBIG-induced passive immunoprophylaxis.[30,35,36] The second and third doses of the vaccine are given at one and six months.[30,34] In endemic regions of HBV infection active immunization with three or four doses of vaccine without HBIG is immunogenic in 90% of neonates born to HBV e antigen-negative carrier mothers or non-carrier mothers.[30,37] In regions in which the majority of the infections that become chronic are acquired a little later in childhood by the horizontal route, the first dose of the vaccine can be given slightly later and without passive immunization. Three injections are normally given. The aim of all vaccine programmes should be to reach vaccine coverage levels of at least 95%.[38]

Novel HBV vaccine strategies are being explored, including the use of epidermal powder immunization[39] and oral administration of HBsAg-transgenic plants.[40,41]

The introduction of HBV vaccine into the EPI in most countries and the beneficial effect this has already achieved in reducing viral carriage rates and the occurrence of HCC in vaccinated children is undoubtedly the most promising and far-reaching development in the prevention of this tumor. HBV vaccine is the only vaccine currently in use that prevents cancer, but its success gives promise that other virally-induced cancers will in future be preventable.

- ***Immunization against hepatitis C virus infection:*** Despite extensive research over many years into the development of a vaccine against HCV, there appears to be little likelihood of such a vaccine becoming available in the near future. Difficulties impeding the development of this vaccine include the extreme variability of the genomic structure of the virus, especially in the hypervariable region, the large number of quasispecies in the blood of infected individuals at any one time, and the lack of evidence for an effective neutralizing antibody against the virus.

  Because immunization against HBV is still not universally practiced and the full beneficial effects of immunization against HBV on the occurrence of HCC will not be felt for many years, and there is still no early prospect of a vaccine against HCV, other methods of preventing the spread of these viruses must continue to be rigidly enforced in an attempt to prevent HCC induced by these viruses.

Other forms of primary prevention against hepatitis C and B virus infections

These forms of primary intervention are potentially more effective in preventing HCV-related than HBV-related HCC. Persistent infection with HCV occurs predominantly in adulthood[12,13,42] by routes that are, in the absence of vaccination, more amenable to intervention than those responsible for the great majority of the chronic HBV infections acqui-

red in early childhood in endemic regions of the virus.[7,8]

Despite recent advances in treating patients with chronic HCV infection with anti-viral drugs, the overall impact of therapy is relatively small because the majority of chronically infected individuals are unaware that they are infected.[43] Consequently, prevention of spread of, and infection with, this virus by means other than vaccination, as well as anti-viral treatment, will continue to be an important strategies for the foreseeable future. Efforts to prevent infection should focus both on identifying persons at increased risk of HCV infection and providing them with counselling and testing for the presence of the virus, and on reducing the incidence of new infections and the risk of progression of these infections to chronic liver disease. The following practices should be introduced on as wide a scale as possible.[43]

- ***Safe injection practices:*** These are based on education of medical, paramedical, and dental practitioners to avoid the use of unnecessary injections and to improve the safety of their injection and infusion techniques. The latter includes the rigid adherence to the use of needles and syringes on a single occasion only or, if this is not possible, the unfailing use of fool-proof methods of sterilization of needles or syringes that have to be re-used. Also important are the avoidance of, or correct use of, multi-dose vials, and lessening the risk of nosocomial infections resulting from 'needle-stick' injuries by the proper disposal of used needles and, and whenever possible, the use of 'disposal-proof 'needles.
  Preventing HCV and HBV infections in illicit drug users remains a difficult and sometimes contentious issue. Changes in injection practices that will minimise sharing of contaminated equipment by providing needle and syringe exchange programs (which should include exchange not only of needles and syringes but also all the other drug paraphanalia) on as wide a scale as is possible is a pivotal part of any program to prevent infection spread of HCV or HBV among intravenous drug addicts.
- ***Screening of donated blood for the presence of hepatitis viruses:*** Transfusion-associated hepatitis C and B virus infections have been virtually eliminated in industrialized countries by screening of all donated blood with very sensitive assays for detecting these viruses. Regrettably, screening of donated blood for blood-borne viruses is not performed in many resource-poor countries, especially in sub-Saharan Africa (where as many as 45% of blood transfusions are estimated to be unscreened).[35] Rectifying this hazardous practice is an essential step in preventing HCV-induced HCC and to a lesser extent HBV-induced HCC in these countries.
- ***The rational use of viral inactivation steps in the manufacture of blood products.***
- ***Passive immunization:*** Passive immunization with HBIG is useful in preventing transmission of HBV, but it is expensive and its effect is of limited duration. The value of immune globulin in preventing HCV infections has still to be ascertained.
- ***Anti-viral agents:*** Treatment with currently used anti-viral agents has limited efficacy in the sustained eradication of hepatitis B and C viruses, and so achieves relatively little in preventing the spread of these viruses. Nevertheless, in those patients with HCV or HBV infection who respond to treatment with anti-viral drugs the risk of HCC development is reduced or delayed (see *Tertiary Prevention*).

Prevention of exposure to aflatoxin B_1

Contamination of staple foodstuffs by AFB_1 does not occur in industrialized countries because those foodstuffs that might be affected are screened for their aflatoxin content by governmental agencies and do not enter the commercial market if unacceptably high levels are found. The problem occurs in resource-poor countries where regulations to control exposure are either non-existent or unenforceable in practice, and where the crops are consumed by the subsistence farmer's family and neighbors and are sold locally or regionally without ever coming under the scrutiny of a governmental agency. It is estimated that about 4.5 billion of the world's population are exposed to aflatoxins. Because contamination by *Aspergillus* species takes place both during growth of the crops and as a result of their improper storage, attempts at primary prevention must be directed at minimising both sources of fungal contamination.[17,18,44,45]

One possible intervention is to alter agricultural practices in regions of high dietary AFB_1 intake by replacing crops that are highly susceptible to fungal contamination with others, such as rice, at lower risk. This approach has been successfully used in one limited study in the People's Republic of China when a change to a rice-based diet resulted in an

appreciable decrease in AFB_1 intake.[17,18] Unfortunately, for most communities in resource-poor countries a change in diet is not feasible. Relatively simple pre-harvest prevention could involve spraying of the crops with fungicides and, because damaged plants are more susceptible to fungal contamination, increasing the resistance of the plants to fungal infection by ensuring adequate irrigation and spraying with insecticides.[17,18,44,45] In the longer term contamination of growing crops might be prevented by the introduction of non-aflatoxigenic strains of *Aspergillus* to compete with the aflatoxin-producing strains, or by genetically engineering foodstuffs that are resistant to infection with *Aspergillus* species. These methods are likely, however, not to be affordable or feasible in those countries with the greatest need to prevent dietary exposure to AFB_1.

The likelihood of contamination during storage is increased by excessive moisture and damage to the crops. Methods of combating this include sun drying of the crops before storage and drying on cloth rather than directly on the earth; removal of visibly mouldy plants by hand sorting; well-ventilated, rainproof storage facilities; storage in jute rather than plastic sacks and in wooden containers rather than on the earth; and the use of insecticides to control insect damage and fungicides to prevent spread of fungal spores.[17,18] A study confirming the effectiveness of post -harvest intervention in significantly reducing AFB_1 intake has been performed in a rural region of Guinea.[45] For these interventions to be successful on a wide scale in resource-poor countries will require education of subsistence farmers in their use and the provision of the means to improve storage facilities, as well as monitoring of levels of contamination.

Prevention of dietary iron overload

Dietary iron overload has virtually disappeared from urban Black Africans as a result of a change in their drinking habits from home-brewed traditional beverages with a high iron content to commercially available iron-free types of alcohol. However, the pattern of alcohol consumption in rural areas remains largely unchanged. Attempts at intervention will require education about the health hazards of alcohol brewed in iron drums or pots, backed up by the provision of suitably-sized aluminium or other iron-free containers in which to prepare the beverage. Such a program has yet to be attempted on a large scale.

Prevention of exposure to blue-green algae and microcystins

Since 1973 the government of the People's Republic of China has been urging rural populations to drink deep-well water.[46] This has resulted, for example in Qidong county, in 80% of the population now drinking deep-well water compared with only 20% in the 1970s.[46] In addition, in some regions the drinking water is treated by granular- activated carbon filtration. The effect of these interventions on the occurrence of HCC has yet to be published.

Secondary prevention

Secondary prevention of HCC, defined as interfering with the metabolism of a carcinogen or preventing it from reaching its target or interacting with tissue nucleophiles, especially DNA, currently provides a limited number of opportunities only. These include early treatment of acute hepatitis C with antiviral agents to prevent progression to chronic hepatitis,[47-49] modulating phase I and II metabolism of AFB_1, and early diagnosis and treatment of some inherited metabolic diseases to prevent the complicating tumor. In addition, some of the genetic and epigenetic changes involved in the complex pathogenesis of HCC have been unravelled and modification of these may lend themselves in time to secondary prevention of the tumor. For example, secondary prevention of HCC in individuals already chronically infected with HBV might in the future be accomplished by preventing HBV replication or the expression of the HBV x gene, which has been incriminated in the pathogenesis of HBV-induced HCC, with appropriately designed small interfering ribonucleic acids or ribozymes.[50] Another future possibility might be the use of DNA vaccination, which has been shown in animal models to induce antibodies against HBsAg/anti-HBs.[51,52]

Chemoprevention may make use of natural or synthetic chemicals to block, retard, or reverse the carcinogenic process. Effective strategies need to be safe, inexpensive, and mechanistically simple. Some preliminary evidence is available that chemoprevention might play a role in preventing AFB_1-induced HCC.

- ***Chemoprevention of aflatoxin B_1- induced hepatocellular carcinoma:*** Chemoprevention of AFB_1-induced malignant transformation is based on the principle of attenuating the consequences of currently unpreventable dietary

exposure to the toxin. This is attempted by modulating the balance between metabolic activation and detoxification of the reactive AFB_1 metabolites, AFB_1-8,9, *exo* epoxide and AFB_1-FABY in particular. AFB_1-induced malignant transformation in experimental animals can be inhibited by many chemopreventive agents and in a variety of ways. However, very few of these agents are suitable for use in humans. One such agent is chlorophyllin.

- **Chlorophyllin.** Sodium copper chlorophyllin, a water soluble derivative of natural chlorophylls, is a potent anti-carcinogen in a number of experimental models,[53] including AFB_1-induced HCC. Chlorophyllin acts as an "interceptor molecule", forming tight molecular complexes with a number of chemical carcinogens, including aflatoxins, thereby reducing their bioavailability and hence their carcinogenic capability.[53] It also acts as an antioxidant[54] and is a potent inhibitor *in vitro* of cytochrome p450 enzymes involved in the bioactivation of several environmental carcinogens.[53,54] Chlorophylin is most effective if given in molar excess to the carcinogen at or around the time of exposure.[55]

  A single randomised, double-blind, placebo-controlled trial in Qidong county, the People's Republic of China, has thus far been conducted.[56] This showed that chlorophyllin, administered three times a day for four months, caused a 55% reduction in the median level of urinary excretion of AFB_1-*N* ^{7}-gua when compared with placebo. No toxic side effects were observed and compliance to the drug was good. Further trials are needed to ascertain whether the long-term administration of this drug will be feasible and safe. Supplementation of the diet with foods that are rich in chlorophylls, such as spinach and other leafy green vegetables, might be a more practical alternative.
- **Oltipraz.** A second approach is to modify the detoxification pathway of AFB_1 in such a way as to render its reactive metabolite innocuous. The anti-schistosomal drug, oltipraz (a substituted 1,2-dithiole-3-thione) is structurally similar to the dithiolethiones found in cruciferous vegetables that may play a role in cancer prevention.[18,57] Oltipraz is a potent inducer of the expression of the phase II detoxifying enzyme, glutathione-S-transferase, and also regulates the transcription of genes encoding other conjugating or antioxidative enzymes, and might therefore be effective in the secondary prevention of AFB_1-induced HCC.[58,59] KEAP1 sequesters NRF2 in the cytoplasm by binding to its amino-terminal regulatory domain.[57] Treatment with oltipraz disrupts the interaction between KEAP1 and NRF2, allowing NRF2 to translocate to the nucleus, where it forms heterodimers with small MAF-family proteins to activate the expression of glutathione-S-transferase and other genes,[18] enhancing the phase II inactivation of the AFB_1-8,9 epoxides. More recent studies of the pharmacodynamic effects of oltipraz have shown that the drug also has an inhibitory effect on certain phase 1 enzymes, including CYP3A4 and CYP1A2.[60] It therefore reduces the activation of AFB_1 to AFB_1-8,9-epoxides.

  A randomised, placebo-controlled, double-blind trial of oltipraz conducted in adults with detectable serum levels of aflatoxin;albumin adduct in Qidong county showed a 2.6-fold increase in the urinary excretion of the AFB_1- 8,9-*exo*-epoxide metabolite, AFB_1-mercapturic acid, and lesser increases in excretion of other AFB_1 biomarkers.[61] In another study, one month of therapy with weekly oltipraz administration led to a significant decrease in aflatoxin M_1 excretion in the urine and sustained low dose oltipraz increased conjugation of AFB_1.[62] However, because of cost and safety considerations, it is doubtful whether oltipraz could be used on a wide scale in the secondary prevention of HCC.
- ***Other chemoprevention possibilities:*** A decrease in the risk of HCC correlates with an increased consumption of leafy, green vegetables.[63] Plants belonging the family *Cruciferae* and the genus Brassica (including broccoli, cauliflower, and Brussel sprouts) contain large quantities of isothiocyanates, mostly in the form of their glycosinolate precursors. Some of these isothiocyanates have been shown to inhibit tumor formation in rats.[64] Trials of the use of glycosinolates and isothiocyanates from broccoli sprouts in preventing HCC in cohorts of subjects at high risk for the tumor are in progress. However, one such trial in Qidong county, the People's Republic of China, showed no difference in urinary AFB_1-N^7-gua levels between volunteers receiving infusions of broccoli sprouts and those receiving placebo.[64]
- ***Iron storage diseases:*** Whether or not excess hepatic iron is proved to be directly hepatocarcinogenic, 'de-ironing' of patients with HH by repeated venesection would be expected to have a secondary preventive effect against HCC formation, both by reversing the accumulation of iron and preventing the development of fibrosis and

cirrhosis. 'De-ironing' aims to lower the serum ferritin concentration to a normal level and to maintain it there. Studies have shown that removing the excess iron dramatically improves life expectancy, the number of cases of HCC decreases if cirrhosis is not present, and survival improves considerably in those with cirrhosis.[65] This intervention is preferably commenced as soon as the diagnosis of HH is made and should be continued for life.

Family members of patients with HH should be screened on the basis of their percentage transferrin saturation followed, if necessary, by gene testing.

Those found to be positive for HH should have regular prophylactic venesections, most conveniently administered by the local blood transfusion service.

Secondary prevention or reduction of excess hepatic iron in dietary iron overload has not been attempted on a large scale.

Tertiary prevention

Tertiary prevention is defined as preventing precancerous lesions from progressing to cancer. Cirrhosis is a precancerous condition.[14-16] The three most common causes of cirrhosis complicated by the development of HCC are chronic HBV and HCV infections and alcohol abuse. In the first two, long-term suppression of viral replication could be expected to reduce hepatic inflammation and hepatocyte necrosis and proliferation, and hence lessen the risk of progression to malignant transformation.[16,66] IFN has been most widely used in the treatment of both chronic HCV- and HBV-induced diseases, and glycyrrhizin has long been tried in chronic HCV infection in Far Eastern countries. These agents may be regarded as being immunopreventive by functioning as biological response modifiers.

Use of anti-viral agents in preventing hepatitis C virus-induced hepatocellular carcinoma

HCV-related HCC has become an increasingly more common and more important tumor in many industrialized countries. Its development is closely related to the duration and progression of chronic HCV-induced hepatitis and cirrhosis. A large number of studies have shown that the risk of malignant transformation is reduced in those patients who achieve a sustained virologic response.[38,67-76] The risk may also be reduced in some patients who achieve only a sustained biochemical response (normalization of serum transaminase levels)[38,67,71,74,75] or perhaps even no response provided that fibrosis was mild (F1).[69] HCC is more likely to develop in the presence of severe hepatic fibrosis,[74] and the likelihood of IFN preventing HCC formation is proportional to the degree of hepatic fibrosis.[69] Fibrosis has been shown either to progress more slowly or to regress in patients in whom IFN induces a sustained virological response and normalization of serum transaminase levels, indicating that the anti-fibrotic effects of IFN may contribute to its chemoprevention capability. [70,73]

A tertiary preventive effect of IFN is supported by studies that show that this treatment prevents recurrences of HCC after initial surgical resection or ablative treatment.[76,77]

The mechanism or mechanisms by which IFN reduces the risk for HCC are uncertain. Clearance of the virus and reduction in hepatic inflammation and its consequences are obvious factors, but another mechanism may be upregulating the function of tumor suppressor genes.[78] In support of this mechanism is the observation that IFN has a tumor inhibiting effect on HCC cell lines[79]

The combination of pegylated IFN and ribavirin in the treatment of patients with chronic HCV infection has improved the sustained virological clearance rates and might therefore be expected to be further improve the results of anti-viral treatment. A recent meta-analysis shows a positive effect of the drug combination in preventing the development of HCC.[72]

Use of anti-viral agents in preventing chronic hepatitis B virus-induced hepatocellular carcinoma

Recent studies have shown that patients with persistently high levels of HBV replication, as shown by the presence of HBeAg in serum and high viral counts, are at high risk of HCC and that chronic HBV carriers with low serum HBV DNA levels seldom progress to tumor formation.[80-83] Theoretically, anti-viral treatment that results in viral clearance or sustained suppression of HBV replication should prevent the development of HCC. However, in contrast to the many studies of the effect of IFN in preventing the development of HCC in patients with HCV-induced chronic hepatitis or cirrhosis, fewer studies have addressed this issue in patients with HBV-related disease and the findings have been conflicting. Although most of the earlier studies failed

to provide convincing evidence that treating such patients with IFN would lessen the risk of malignant transformation,[67,68,84] recent studies have shown that IFN does lessen the risk.[85-87] In the first of the latter studies, 2.7% of patients treated with IFN developed HCC compared with 13% in those not treated ($p = 0.011$).[84] In the second, HCC developed in 34% of patients without clearance of HBV DNA compared with 8% in those with clearance ($p = 0.026$).[86] These results were confirmed in a recent meta-analysis.[88]

In addition, three recent trials have shown a protective effect of lamivudine treatment in preventing the development of HCC in patients with chronic HBV infection. In the first, a randomised, double-blind, placebo-controlled trial, HCC occurred in 3.9% of Taiwanese patients in the lamivudine-treated group compared with 7.4% in the placebo-treated group (hazard ratio 0.49: $p = 0.047$).[89] In a case-control study, 142 HBeAg-positive lamivudine-treated patients were compared to 124 controls matched for age, HBeAg status, and absence of cirrhosis, and followed for a mean of 89 months.[90] Lamivudine-treated patients had a significantly lower cumulative rate of cirrhosis and/or HCC ($p = 0.005$) than did those not receiving lamivudine. Even those patients who developed the YYMD mutation had a lower rate of cirrhosis and/or HCC ($p = 0.024$).[90] The third case/control study of a large number of Japanese patients with chronic hepatitis B showed that those who received lamivudine treatment had an annual incidence of HCC of 0.4%/patient compared with 2.5%/patient in the control group.[91] These results were confirmed in a recent meta-analysis.[88] The main disadvantage of the long-term administration of lamivudine is the emergence of resistant strains of the virus as a result of the development of YMDD mutations. Newer drugs of a similar sort, adefovir and entecavir, have a far lower risk of developing resistant strains, and future trials should examine their efficacy in preventing HCC. In addition, future trials need to further address the questions of who to treat and for how long treatment should be administered.

Glycyrrhizin

Glycyrrhizin, the active principle of licorice, has a chemical structure similar to cortisone. It is composed of one molecule of glycyrretinic acid and two molecules of glucuronic acid. Glycyrrhizin enhances IFN-γ production, has immune modulating activity, and stimulates natural killer cells.[92,93] The compound also has anti-oxidative properties and suppresses hepatic inflammation.[92,93] It has been used for over 35 years in Japan, partly in the form of the Chinese herbal medicine, Sho-saiko-to, of which glycyrrhizin is one of the main ingredients, in the treatment of chronic hepatitis (by far the most common cause of which in Japan is HCV). Daily intravenous injections of glycyrrhizin reduce the levels of the serum aminotransferases in a dose-dependent manner in these patients, although antiviral activity *per se* is not evident.[94] In a prospective trial performed in Japan over 15-20 years, a 2.5-fold lower risk of HCC formation was reported in patients receiving parenteral glycyrrhizin compared to those who did not [88]. HCV-RNA titers did not decrease, and the beneficial effect of the drug was attributed to controlling or retarding necroinflammatory and fibrotic processes in the liver. These conclusions were supported by two further studies.[95,96] In addition, glycyrrhizin reduced the risk of HCC recurrence after surgical resection.[97]

Daily intravenous injections of glycyrrhizin would appear to be a drawback of this approach to prevention.

Another chemopreventive approach is with polyphenols derived from green teas. These agents have been effective in preventing liver tumors in animal models and a clinical study is underway in Guanxi, People's Republic of China.[98]

ABBREVIATIONS

- **HCC:** Hepatocellular carcinoma.
- **HBV:** Hepatitis B virus.
- **HCV:** Hepatitis C virus.
- **NASH:** Non-alcoholic steatohepatitis.
- **AFB_1:** Aflatoxin B_1.
- **AFB_1-N^7-gua:** 8,9-dihydro-8-(N^7-guanyl)-9-hydroxy-AFB_1 DNA adduct.
- **DNA:** Deoxyribose nucleic acid.
- **EPI:** Expanded Program of Immunization.
- **HIV/AIDS:** Human immunodeficiency virus/aquired immunodeficiency disease syndrome.
- **HBsAg:** Hepatitis B virus surface antigen.
- **HBIG:** Hepatitis B virus immune globulin.
- **Anti-HBs:** Antibody to hepatitis B virus surface antigen.
- **AFB_1-FABY:** AFB_1-formamidopyrimidine adduct.
- **HH:** Hereditary hemochromatosis.
- **CYP:** Cytochrome P.
- **NRF2:** Nuclear receptor factor 2.

- **KEAP1:** Kelch-like ECH associated factor 1.
- **MAF:** Macrophage activating factor.
- **IFN:** Interferon-α.

REFERENCES

1. Ferlay J, Bray F, Pisani P, Parkin DM. GLOBOCAN 2000: Cancer Incidence, Mortality and Prevalence Worldwide. Version 1.0. IARC Cancer Base No. 5. International Agency for Research on Cancer: IARC Press, Lyon, 2001.
2. Bosch FX, Ribes J. The epidemiology of primary liver cancer: global epidemiology. In: Viruses and Cancer. Tabor E (ed.). Amsterdam: Elsevier; 2002, p. 1-16.
3. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics 2002. *CA Cancer J Clin* 2005; 55: 74-108.
4. Bosch FX, Ribes J, Diaz M, Cleries R. Primary liver cancer: worldwide incidence and trends. *Gastroenterology* 2004; 127: S5-S16.
5. Yu MC, Yuan MJ, Govindarajan S, Ross SK. Epidemiology of hepatocellular carcinoma. *Can J Gastroenterol* 2000; 14: 703-9.
6. World Health Organization (WHO) (2004). Hepatitis vaccines. *Wkly Epidem Rev* 79; 255-63.
7. Stevens CE, Szmuness W. Vertical transmission of hepatitis B and neonatal hepatitis B. In: Viruses and the Liver. Bianchi L, Gerok W, Sickinger K, Stalder GA (eds.). Lancaster, UK: MTP Press. 1980: 71-4
8. Botha JA, Ritchie MJJ, Dusheiko GM, Mouton HWK, Kew MC. Hepatitis B virus carrier state in Black children in Ovamboland: Role of perinatal and horizontal infection. *Lancet* 1984; 2: 1209-12.
9. Atkinson W, Mamborsky J, McIntyre L, Wolfe S. Hepatitis B. In: Epidemiology and Prevention of Vaccine-preventable Diseases. 8th. Ed. Washington DC, WA: Public Health Foundation; 2005, p. 151-212.
10. Beasley RP, Hwang L-Y, Lin CC, Chien CS. Hepatocellular carcinoma and HBV: A prospective study of 22,707 men in Taiwan. *Lancet* 1981; 22: 1129-33.
11. International Agency for Research on Cancer. Hepatitis Viruses. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 59. Lyon, France: IARC Press; 1994.
12. Kew MC. Hepatitis C virus and hepatocellular carcinoma in developing and developed countries. *Vir Hepatit Rev* 1998; 4: 259-69.
13. Kiyosawa K, Umemura T, Ichijo T, Matsumoto A, Yoshizawa K, Gad A, Tanaka E. Hepatocellular carcinoma: Recent trends in Japan. *Gastroenterology* 2004; 127: S17-S26.
14. Kew MC. The role of cirrhosis in hepatocarcinogenesis. In: Liver Cell Carcinoma. Bannasch P, Keppler D, Weber G. (eds.). Dordrecht: Kluwer Academic Publishers; 1989. p. 37-46.
15. Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004; 127: S35-S50.
16. Kew MC. Hepatocellular carcinoma. Molecular biology and genetics. In: Gastrointestinal Oncology. 2nd. Ed. Kelsen DP, Daly JM, Kern SE, Levin B, Tepper JE, Van Cutsem E (eds.). Philadelphia: Lippincott, Williams and Wilkins; 2008, p. 405-18.
17. Wild CP, Gong YY. Mycotoxins and human disease: a largely ignored global health issue. *Carcinogenesis* 2010; 31: 71-82.
18. Sudakin DL. Dietary aflatoxin exposure and chemoprevention of cancer: a clinical review. *J Toxicol Clin Toxicol* 2003; 41: 195-204.
19. Kew MC. Synergistic interaction between aflatoxin B1 and hepatitis B virus in hepatocarcinogenesis. *Liv Internat* 2003; 23: 1-5.
20. Niederau C, Fischer R, Sonnenberg A, Stremmel W, Trampisch HJ, Strohmeyer G. Survival and causes of death in cirrhotic and non-cirrhotic patients with hereditary hemochromatosis. *N Engl J Med* 1985; 313: 1256-62.
21. Francanzani AL, Conte D, Fraquelli M, Roncalli M, Bonelli N, Borzio F, Maraschi A, et al. 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* 2001; 33: 647-51.
22. Bothwell TH, Bradlow B. Siderosis in the Bantu: a combined histopathological and chaemical study. *Arch Pathol* 1960; 70: 279-92.
23. Gordeuk VR. African iron overload. *Sem Hematol* 2002; 39: 263-9
24. Isaacson C, Seftel HC, Keeley KJ, Bothwell TH. Siderosis in the Bantu: the relationship between iron overload and cirrhosis. *J Lab Clin Med* 1981; 58: 845-85
25. Mandishona E, MacPhail AP, Gordeuk VR, Kedda MA, Paterson AC, Rouault TA, Kew MC. Dietary iron overload as a risk factor for hepatocellular carcinoma in Black Africans. *Hepatology* 1998; 27: 1563-6.
26. Asare GA, Paterson AC, Kew MC, Khan S, Mossanda KS. Iron-free neoplastic nodules and hepatocellular carcinoma without cirrhosis in Wistar rats fed a diet high in iron. *J Pathol* 2006; 208: 82-90
27. Yu SZ, Chen G. Blue-green algae toxins and liver cancer. *China J Cancer Res* 1994; 6: 9-17.
28. Zhou TL, Yu SZ. Laboratory study on the relationship between drinking water and hepatoma: Quantitative evaluation using GGT method. *China J Prev Med* 1990; 234: 203-5.
29. Lavanchy D. Worldwide epidemiology of HBV infection, disease burden, and vaccine prevention. *J Clin Virol* 2005; 34 (Suppl), S1-S3.
30. Chang MH. Changing incidence of hepatocellular carcinoma among children following universal hepatitis B immunization. *Liver Linternat* 2003; 23 ; 309-14.
31. Chang M-H, Shau WY, Chen CJ, Wu TC, Kong NS, Liang DC, Hsu MH, et al. The effect of universal hepatitis B vaccination on hepatocellular carcinoma rates in boys and girls. *JAMA* 2000; 284: 3040-2.
32. Lee CL, Hsieh KS, Ko YC. Trends in the incidence of hepatocellular carcinoma in boys and girls in Taiwan after large-scale hepatitis B vaccination. *Cancer Epidemiol Biomarkers Prev* 2003; 12: 57-9.
33. Chang MH, You SL, Chen CJ, Liu CJ, Lee CM. Lin SM. Chu HC, et al. Decreased incidence of hepatocellular carcinoma in hepatitis B vaccines: a 20 year follow-up. *J Natl Cancer Inst* 2009; 101: 1348-55.
34. Kane MA. Global control of primary hepatocellular carcinoma with hepatitis B vaccine: The contributions of research in Taiwan. *Cancer Epidemiol Biomarkers Prev* 2003; 12: 2-3.
35. World Health Organization (WHO) Hepatitis B Fact Sheet No. 204 (revised October 2000). 2000. Available at www.who.int.
36. Chang MH, Chen THH, Tsu, Wu TC, Kong S, Liang DC, Ni YH, et al. Prevention of hepatopcellular carcinoma: the effect and problems. *Clin Cancer Res* 2005; 11: 7953-7.
37. Prozesky OW, Stevens CE, Szmuness W, Rolka H, harley EJ, Kew MC, Scholtz JE, et al. Immune response to hepatitis B vaccine in newborns. *J Infect* 1983; 7 (Suppl): S53 -S55.
38. PAHO (Pan American Health Organization). Health in the Americas. Washington, DC, PAHO 2002.
39. Chen D, Weis KF, Chun Q, Erickson C, Endres R, Lively CR, Osorio J, et al. Epidermal powder immunization induces both cytotoxic T-lymphocyte and antibody responses to

protein antigens of influenza and hepatitis viruses. *J Virol* 1999; 75: 11630-40.
40. Kapusta J, Modelska A, Figlerowicz M, Pniewski T, Letellier M, Lisowa W, Yusibov V, Mason HF. A plant-derived edible vaccine against hepatitis B virus. *FASEB J* 1999; 13: 1796-9.
41. Richter LJ, Thanavla Y, Arntzsen CJ, Mason HS. Production of hepatitis B surface antigen in transgenic plants for oral immunization. *Nat Biotechnol* 2000; 18: 1167-71.
42. Heathcote EJ. Prevention of hepatitis C virus-related hepatocellular carcinoma. *Gastroenterology* 2004; 127: S294-S302.
43. Kew MC, Francois G, Lavanchy D, Margolis H, van Damme P, Grob P, Hallauer J, et al. Prevention of hepatitis C virus infection. *J Med Virol* 2004; 11: 198-205.
44. Kensler TW, Egner PA, Wang JB, Zhu YR, Zhang BC, Lu PX, Chen JG, et al. Chemoprevention of hepatocellular carcinoma in aflatoxin endemic areas. *Gastroenterology* 2004; 127: S310-S318.
45. Turner PC, Sylla A, Gong YY, Diallo MS, Sutcliffe AE, Hall AJ, Wild CP. Reduction in exposure to carcinogenic aflatoxins by post harvest intervention measures in west Africa: a community-based intervention study. *Lancet* 2005; 365: 1950-6.
46. Yu SZ. Primary prevention of hepatocellular carcinoma. *J Gastroenterol Hepatol* 1995; 10: 674-82.
47. Jaeckel E, Cornberg M, Wedemeyer H, Santantonio T, Mayer J, Zankel M, Pastore G, et al. Treatment of acute hepatitis C with interferon a-2a. *N Engl J Med* 2001; 345: 1452-7.
48. Gerlach JT, Diepolder HM, Zachoval R, Gruener NH, Jung MC, Ulsenheimer A, Schraut WW, et al. Acute hepatitis C: high rate of both spontaneous and treatment-induced viral clearance. *Gastroenterology* 2003; 125: 80-8.
49. Nomura H, Dou S, Tanimoto H, Nagahama T, Kimura Y, Hatashi J, IshibashiA, et al. Short-term interferon a therapy for acute hepatitis C: a randomized control trial. *Hepatology* 2004; 39: 1213-9.
50. Arbuthnot P, Carmona S, Ely A. Exploiting the RNA interference pathway to counter hepatitis B virus replication. *Liv Internat* 2005; 25: 9-15.
51. Prince AM, Whalen R, Brotman B. Successful nucleic acid-based immunization of new-born chimpanzees against hepatitis B virus. *Vaccine* 1997; 15: 916-9.
52. Michel ML, Mancini-Bourgine M. Therapeutic vaccination against chronic hepatitis B virus infection. *J Clin Virol* 2005; 34 (Suppl. 1): S108-S114.
53. Dashwood R, Negishi T, Hayatsu H, Breinholt V, Hendriks J, Baily G. Chemopreventive properties of chlorophylls towards aflatoxin B1 : a review of the antimutagenicity asd anticarcinogenicity data in Rainbow trout. *Mutation Res* 1998; 399: 245-53.
54. Kamat JP, Boloor KK, Devasgayan TP. Chlorophyllin as an effective anti-oxidant against the membrane damage in vitro and ex vivo. *Biochim Biophys Acta* 2000; 1487: 113-27.
55. Morel F, Fardel O, Meyer DJ, Langouet S, Gilmore KS, Meunier B, Tu CP, et al. Preferential increase of glutathione-S-transferase class ? transcripts in cultured human hepatocytes by phenobarbital, 3.methylcholanthrene and dithiolethiones. *Cancer Res* 1993; 53: 231-4.
56. Egner PA, Wang J-B, Zu YR, Yhang BC, Wu Y, Zhang QY, Qian GS, et al. Chlorophyllin intervention reduces aflatoxin-DNA adducts in individuals at high risk of liver cancer. *Proc Natl Acad Sci USA* 2001; 98: 14601-6.
57. Gupta E, Olepade OI, Ratain MJ, Mick R, Baker TM, Berzin FK, Benson AB, et al. Pharmacokinetics and pharmacodynamics of oltipraz as a chemopreventive agent. *Clin Cancer Res* 1995; 1: 1133-8.
58. Itoh K, Wakabayashi N, Kotoh H, Ishii T, Iganashi, K, Enge JP, Yamamoto M, et al. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the aminoterminal Nrf2 domain. *Genes Dev* 1999; 13: 76-86.
59. Langouet S, Coles B, Morel F, Becquemont L, Beaume P, Guengerich FP, Kettere B, et al. Inhibition of CYP1A2 and CYP3A4 by oltipraz results in the reduction of aflatoxin B1 metabolism in human hepatocytes in primary culture. *Cancer Res* 1995; 55: 5574-9.
60. Sofowora GG, Choo EF, Mayo G, Shyr Y,Wilkinson GRl. In vivo inhibition of human CYP2A2 activity by olipraz. *Cancer Chemother Pharmacol* 2001; 47: 505-9.
61. Kensler TW, He X, Otieno M, Eggner P, Jacobson LP, Chen B, Wang JS, et al. Oltipraz chemoprevention trial in Qidong, People's Republic of China: study design and clinical outcomes. *Cancer Epidemiol Biomarkers Prev* 1997; 6: 257-65.
62. Wang JS, Shen X, He X, Zhu YR, Zhang BC, Wang JB, Qian GS, et al. Protective alterations in phase 1 and 2 metabolism of aflatoxin B1 by oltipraz in residents of Qidong , People's Republic of China. *J Natl Cancer Inst* 1999; 91: 347-54.
63. Fahey JW, Zhang Y, Talalay P. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc Natl Acad Sci USA* 1997; 94: 10367-72.
64. Kensler TW, Chen JG, Egner PA, Fahey JW, Jacobson LP, Stephenson KK, Ye L, et al. Effects of glucosinolate-rich broccoli sprouts on urinary levels of aflatoxin-DNA adducts and phenanthrene tetraols in a randomised clinical trial in He Zuo township, Qidong, People's Republic of China. *Cancer Epidemiol Biomark Prev* 2005; 14: 2605-13.
65. Niederau C, Fischer R, Purschel A, Stremmel W, Hassinger D, Strohmeyer G. Long-term survival in patients with hereditary hemochromatosis. *Gastroenterology* 1996; 110: 1107-19.
66. Kew MC, Popper H. Relationship between hepatocellular carcinoma and cirrhosis. *Semin Liver Dis* 1984; 1: 59-67.
67. Camma C, Giunta M, Andreone P, Craxi A. Interferon and prevention of hepatocellular carcinoma in viral cirrhosis: an evidence-based approach. *J Hepatol* 2001; 34: 593-602.
68. Tabor E. Interferon for preventing and treating hepatocellular carcinoma associated with hepatitis B and C viruses. *Dig Liv Dis* 2003; 35: 297-305.
69. Soga K, Shihasaki K, Aoyagi Y. Effect of interferon on incidence of hepatocellular carcinoma in patients with chronic hepatitis C. *Hepatogastroenterology* 2005; 52: 1154-8.
70. Omata M, Yoshida H. Resolution of liver cirrhosis and prevention of hepatocellular carcinoma by interferon therapy against chronic hepatitis C. *Scand J Gastroenterol* 2003; Suppl 237: 47-51.
71. Ikeda K, Saitoh S, Kobayashi M, Suzuli Y, Suzuki F, Tsubota A, Arase Y, et al. Long-term interferon for one year or longer reduces the hepatocellular carcinogenesis rate in patients with liver cirrhosis caused by hepatitis C virus: A pilot study. *J Gastroenterol Hepatol* 2001; 16: 406-15.
72. Singal AK, Singh A, Jaganmhohan S, Gutura P, Mummadi R, You YF, Sood GK. Antiviral therapy reduces risk of hepatocellular carcinoma in patients with hepatitis C virus related cirrhosis. *Clin Gastroenterol Hepatol* 2010; 8: 192-9.
73. Takimoto M, Ohkoshi S, Ichida T, Takeda Y, Namoto M, Asakura H, Nailo A, et al. Interferon inhibits progression

of liver fibrosis and reduces the risk of hepatocarcinogenesis in patients with chronic hepatitis C. *Dig Dis Sci* 2002; 47: 170-76.
74. Craxi A, Camma C. Prevention of hepatocellular carcinoma. *Clin Liver Dis* 2005; 9: 329-46.
75. Arase Y, Ikeda K, Suzuki F, Suzuki Y, Kobayashi M, Akuta N, Hosaka t, et al. Interferon-induced biochemical response reduces hepatocarcinogensis in hepatitis C virus infection. *J Med Virol* 2007; 79: 1485-90.
76. Kubo S, Nishiguchi S, Hirohashi K, Tanaka H, Shuto T, Yamazaki O, Shiomi S, et al. Effects of long-term postoperative interferon-α therapy on intrahepatic recurrence after resection of hepatitis C virus-related hepatocellular carcinoma. *Ann Intern Med* 2001; 134: 963-67.
77. Miyake Y, Takaki A, Iwasaki Y Yamamoto K. Meta-analysis: Interferon-á prevents the recurrence after curative treatment of hepatitis C virus-related hepatocelluar carcinoma. *J Viral Hepat* 2010; 17: 287-92.
78. Melen K, Keskinen P, Lehtonen A, Julkinen I. Interferon-induced gene expression and signalling in human hepatoma cell lines. *J Hepatol* 2000; 33: 764-72.
79. Yamashita Y, Koike K, Takaoki M, Matsuda S. Suppression of HBsAg production in PLC/PRF/5 human hepatoma cell line by interferons. *Microbiol Immunol* 1988; 32: 1119-26.
80. MacMahon BJ, Holck P, Bulkow L, Snowball M. Serological and clinical outcomes in 1536 Alaska natives chronically infected with hepatitis B virus. *Ann Intern Med* 2001; 135: 759-68.
81. Yang HI, Lu SN, Liaw YF, You SL, Sun CA, Wang LY, Hsiao CK, et al. Hepatitis B e antigen and the risk for hepatocellular carcinoma. *N Engl J Med* 2002; 347: 168-74.
82. Ikeda K, Saitoh S, Suzuki Y, Kobayashi M, Tsubota A, Fukudu M, Koida I, et al. Interferon decreases hepatocellular carcinogenesis in patients with cirrhosis caused by hepatitis B virus: A pilot study. *Cancer* 1998; 82: 827-35.
83. Yuen MF, Hui CK, Cheng CC, Wu CH, Lai YP, Lai CL. Long-term follow-up of interferon-a treatment in Chinese patients with chronic hepatitis B infection: The effect of hepatitis B e antigen seroconversion and the development of cirrhosis-related complications. *Hepatology* 2001; 34: 139-45.
84. Lok A. Prevention of hepatitis B virus-related hepatocellular carcinoma. *Gastroenterology* 2004; 127: S303-9.
85. Lin SM, Yu ML, Lee CM, Chen RM, Sheen IS, Chu CM, Liaw YF. Interferon therapy in HBeAg-postive chronic hepatitis reduces progression to cirrhosis and hepatocellular carcinoma. *J Hepatol* 2007; 46: 45-52.
86. Ikeda K, Kobayashi M, Saitoh S, Someya T, Hasaka T, Akuta H, Suzuki Y, et al. Significance of hepatitis B virus DNA clearance and early prediction of hepatocellular carcinogenesis in patients with cirrhosis undergoing interferon therapy: long-term follow up of a pilot study. *J. Gastroenterol. Hepatol.* 2005; 20: 95–102.
87. Yang WF, Zhao W, Zong WD.. Interferon therapy in chronic hepatitis B reduces progression to cirrhosis and hepatocellular carcinoma: a meta-analysis. *J Vir Hepat* 2009; 16: 265-71.
88. Sung JJ, Tsoi KK, Wong VW, Chan HL. Meta-analysis: Treatment of hepatitis B infection redices risk of hepatocellular carcinoma. *Aliment Pharmacol Ther* 2008; 28: 1067-77.
89. Liaw YF, Sung JJ, Chow WC, Farrell G, Lee LZ, Nuen H, Tanwandee T, et al. Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* 2004; 351: 1521-31.
90. Yuen MF, Seto WK, Chow DH, Tsui K, Wong PK, Ngai VW, Wong BC, et al. Long-term lamivudine treatment decreases the risk of long-term complications of chronic hepatitis B infection even in patients with advanced liver disease. *Antiviral Ther* 2007; 12: 1295-303.
91. Matsumoto A, Tanaka E, Rokuhara A, Kiyosawa K, Kamada H, Omata M, Okita K, et al. Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic hepatitis B: A multicenter retrospective study of 2795 patients. *Hepatol Res* 2005; 32: 173-84.
92. Abe N, Ebina T, Ishida N. Interferon induction by glycyrrhizin and glycyrrhetinic acid in mice. *Microbiol Immunol* 1982; 26: 535-9.
93. Kimura M, Watanabe H, Abe T. Selective activation of extrathymic T cells in the liver by glycyrrhizin. *Biotherapy* 1992; 5: 167-76.
94. Arase Y, Ikeda K, Murashima N, Chayama K, Tsubota A, Koida I, Suzuki Y, et al. The long-term efficacy of glycyrrhizin in chronic hepatitis C patients. *Cancer* 1997; 79: 1494-500.
95. van Rossum TG, Vulto AG, de Man RA, Brouwer JT, Scalm SW. Glycyrrhizin as a potential treatment for chronic hepatitis C: A double-blind, randomised, placebo-controlled phase 1/2 trial. *Aliment Pharmacol Ther* 1998; 12: 199-205.
96. Kumada H. Long-term treatment of chronic hepatitis C with glycyrrhizin for preventing liver cirrhosis and hepatocellular carcinoma. *Oncology* 2002; 62 (Suppl 1): 94-100.
97. Tarao K, Takemiya S, Tamai S, Sugimasa Y, Ohkawa S, Akaike M, Tanabe H, et al. Relationship between the recurrence of hepatocellular carcinoma and serum aminotransferase levels in hepatectomised patients with hepatitis C virus-associated cirrhosis and hepatocellular carcinoma. *Cancer* 1997; 79: 688-94.
98. Tang L, Tang M, Luo H. Chemoprevention trial of green tea polyphenols in high-risk population of liver cancer: modulation of serum and urinary aflatoxin biomarkers. *Cancer Epidemiol Biomarkers Prev* 2003; 12: 1349.