Screening of Anti-HCV Drugs from Natural Sources

Dr. Hesham I. El-Askary

Prof. of Phytochemistry & Medicinal Plants

Faculty of Pharmacy – Cairo University

- **Member of the *Technical Committee* for Drug registration,**
- **Member of the *scientific Committee* for Herbal products & Dietary supplements,**

Central Administration for Pharmaceutical Affairs,

Ministry of Health & Population

Hepatitis C

WHO has declared:

- hepatitis C as **a global health problem**,

- approx. **3% of the world's population**
  (**170-200 million people**) infected with (a small (+)-RNA virus)

- they are at high risk for **HCV** developing
  * **chronic hepatitis,**
  * **liver cirrhosis,**
  * **hepatocellular carcinoma (HCC).**

In the US, approx. **3 million people**

- are chronically infected with hepatitis C virus (HCV),
- many of whom are still undiagnosed.

In Egypt the situation **is quite worse**.


high risk area
moderate risk area

Chronic hepatitis C can cause:

- **cirrhosis**, a process that takes at least **10 to 20 years**,
  20 % of patients with chronic hepatitis C
- **liver failure**,
  - **one of the most common reasons** for **liver transplantation**
- **liver cancer,**
  - Developed in a **small % of patients** after **20 to 40 years**.
  - Hepatitis C is the cause of about **50% of primary liver cancer** in the developed world.

  - **Men, alcoholics, patients with cirrhosis,**
  - **people over age 40**
  - **those infected for 20 to 40 years**

  are at **higher risk of developing HCV-related liver cancer**.

Hepatitis B (HBV)

HBV as a transmitted infection, is

- **one of the strongest viruses in the world,**
- **is 100 times more infectious than AIDS**
- **10 times more infectious than hepatitis C,**

It is estimated that:

- ~ 2 billion people are infected with HBV,
- 350 million have a chronic infection
- 4 million new acute infections occur each year by HBV.
- HBV is responsible for more than 1 million deaths each year.

The problem of HBV in Egypt **can be traced back the methods of testing for the virus.**

For more than 10 years, **Egypt was testing for HBV using machine known as**

BDNA branching, which:

** **was not sensitive enough,**

detected only HBV in a person if they had more than 700,000 copies of the virus in his body.

** **contributed to the greater spread of HBV,**

as people went about their lives without taking precautions because they thought they didn't have HBV, infecting more people in the process.

The country now

- uses a more advanced machine for testing,
which detects HBV in a body with as few as 300 copies of the virus, it is too late for many.

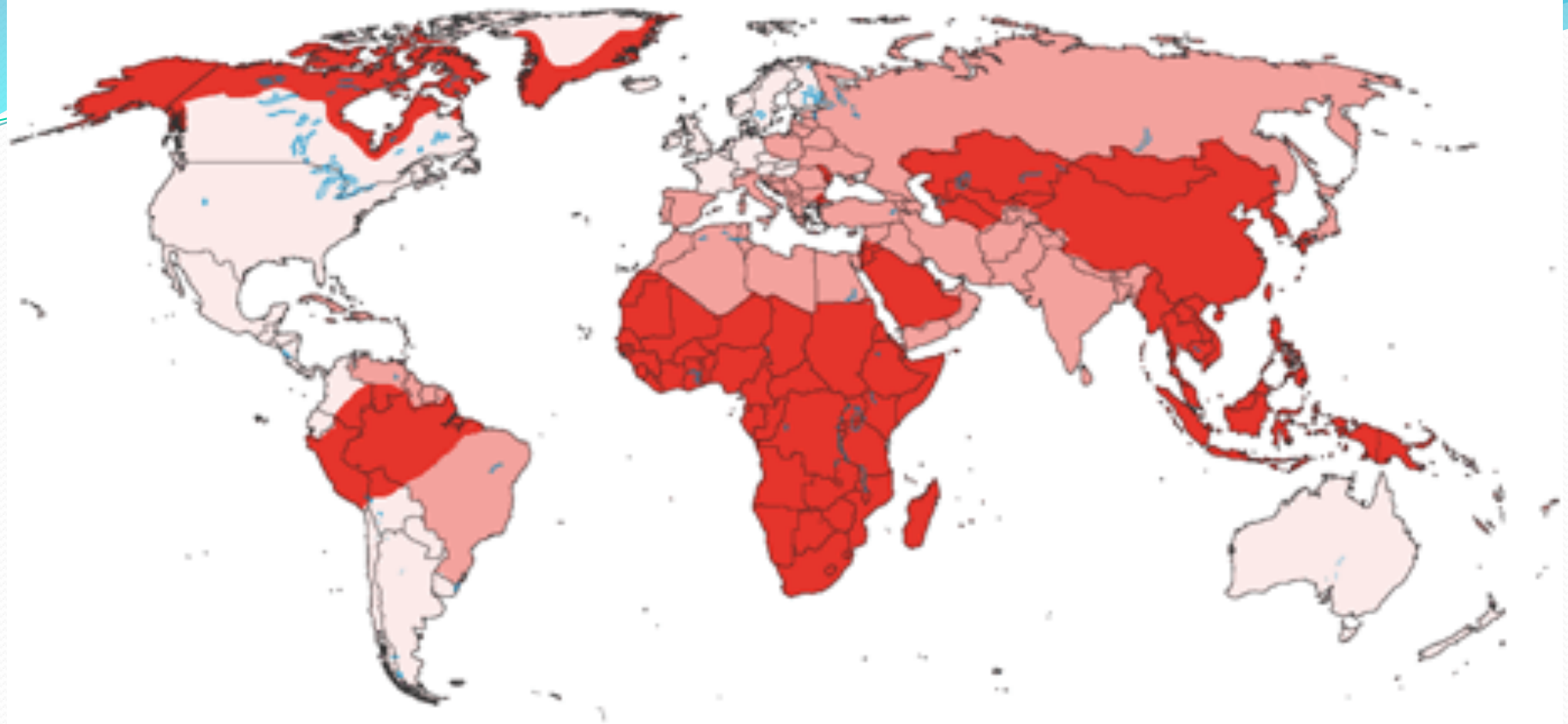
- in 1992 they introduced the HBV vaccine in their national infant immunization programs,
albeit only children under the age of 15 can get the vaccine as part of the program, leaving a huge population of high risk teens susceptible.

What makes HBV so potent

is the fact that

it can **live outside the body on a dry surface for more than a week**, while other viruses, such as **AIDS, die as soon as they leave the body**.

"Unlike the other forms of hepatitis, **HBV can be transmitted through blood, sexual intercourse and through pregnancy,** so a person with HBV can literally transmit it to anyone,



Level of endemicity	% of general population with chronic HBV infection	% of world population
high endemicity	greater than 7%	about 45%
intermediate endemicity	2% to 7%	about 43%
low endemicity	less than 2%	about 12%

The situation of hepatic diseases in Egypt

Updating statistics

- 25 % of the general population has HBV, according to **official statistics in 2006.**

- In fact, of all existent forms of hepatitis, HBV is the most prevalent in Egypt.

-- we actually have close to 2 million people infected with HBV.

the Liver Institute in Menoufia governorate in 1995

- The **highest prevalence rates** of **hepatitis C virus infection in the world** **have been recently reported among Egyptian blood donors and frequent recipients of transfusions and other blood products**.

- This is the first report, however, demonstrating

hepatitis C as the most frequent association with chronic liver disease in Egypt.

Of **1023 patients** referred to the Liver Institute in Menoufia governorate for **evaluation of chronic liver disease**,

752 (73.5%) had **antibodies to hepatitis C** , compared with
168 (16.4%) with **hepatitis B surface antigen.**

Hepatitis C antibody was more common in patients with active **schistosomiasis** and patients without **hepatitis B surface antigenaemia.**

Epidemiology of hepatitis viruses among Hepatocellular carcinoma cases and healthy people in Egypt.

A systematic review and meta-analysis (Lehman & Wilson 2009)

Prevalence of HBV and HCV in Egypt, (in a population-based study)

	HBV	HCV
Healthy population??	6.7 %	13.9 %
Adults	8 %	15.7 %
Children	1.6 %	4.0 %
North of Egypt	4.6 %	15.8 %
South of Egypt	11.7 %	6.7 %
HCC patients*	25.9 %	78.5 %

* **HBV** significantly decreased overtime, While **HCV** did not suggesting a shift in the relative influences of these viruses in HCC etiology in Egypt

Risk Factors and Transmission

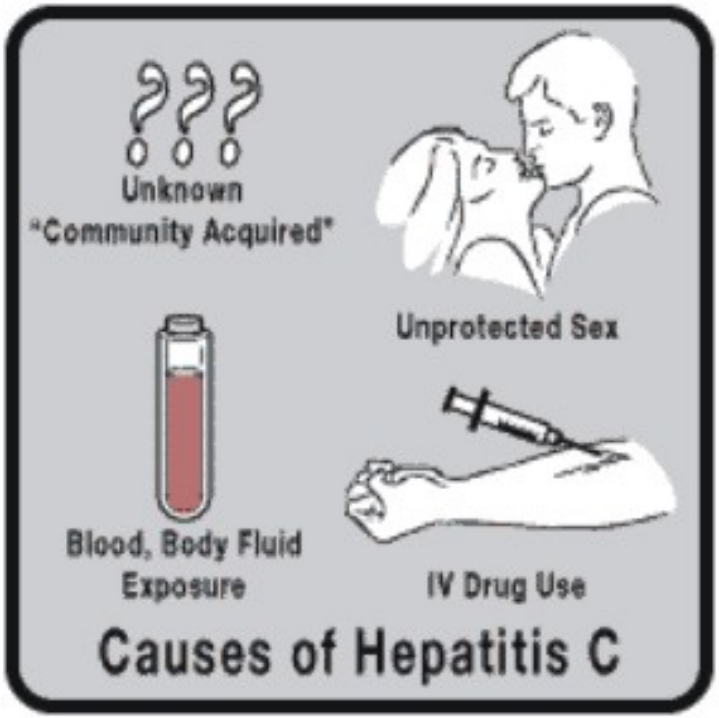
HCV is spread primarily by contact with infected blood and blood products.

With the introduction in **1991** of
- **routine blood screening for HCV antibody**
  -- **improvements in the test in mid-1992,**

transfusion-related hepatitis C has virtually disappeared.

At present, **injection drug use** is the **most common risk factor for contracting** the infection.

However,
some patients who acquire hepatitis C
* do not have a recognized risk factor or
* known exposure to infected blood or to drug use.



The most common risk factors for acquiring hepatitis C

* **Injecting drugs,**
* **A blood transfusion** before June 1992,

  (**sensitive tests for blood screening of anti-HCV**)

* **Receiving clotting factor concentrates**

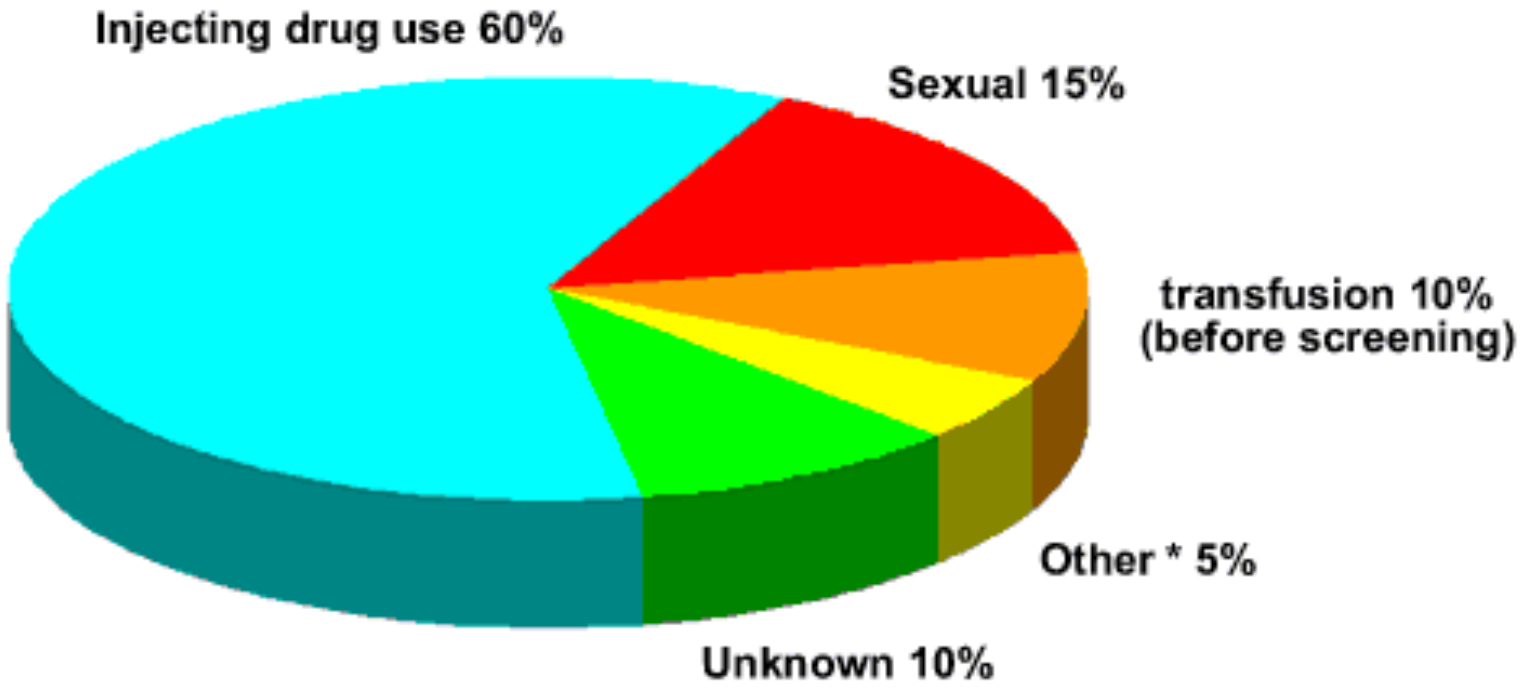
  (such as **anti-hemophilic factor**, before 1987, **effective means to inactive HCV** were introduced)

* **Hemodialysis for kidney failure**
* **Birth to an HCV-infected mother**
* **Suffering a needle-stick accident** from a person with hepatitis C

Other risk factors (with **slightly increased risk** for **hepatitis C**):

* **Sex with someone with hepatitis C**
* **Intranasal use of cocaine** (using shared equipment)

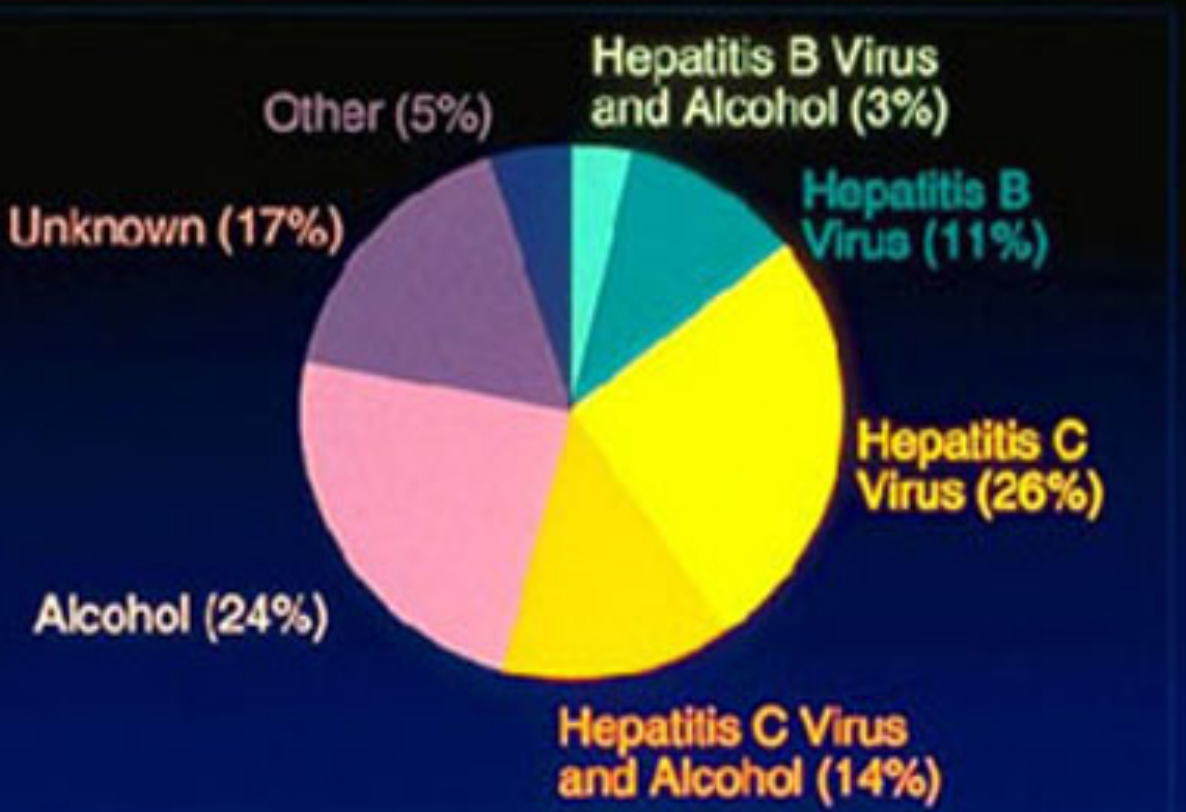
Sources of Infection for Persons with Hepatitis C



*Nosocomial: Health-care work; Perinatal

Source: Centers for Disease Control and Prevention

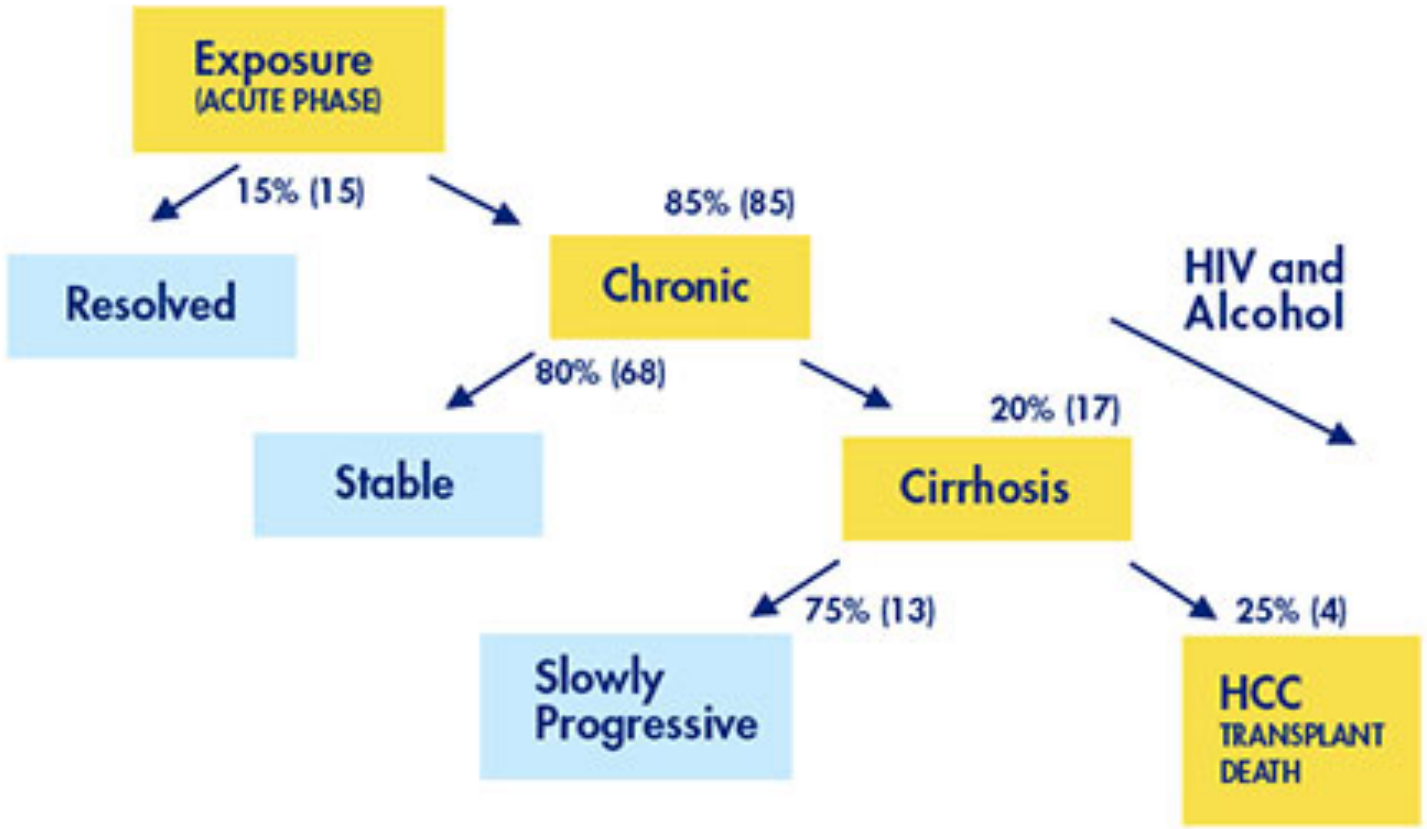
Primary Causes of Chronic Liver Disease*



*Jefferson County, Alabama, USA

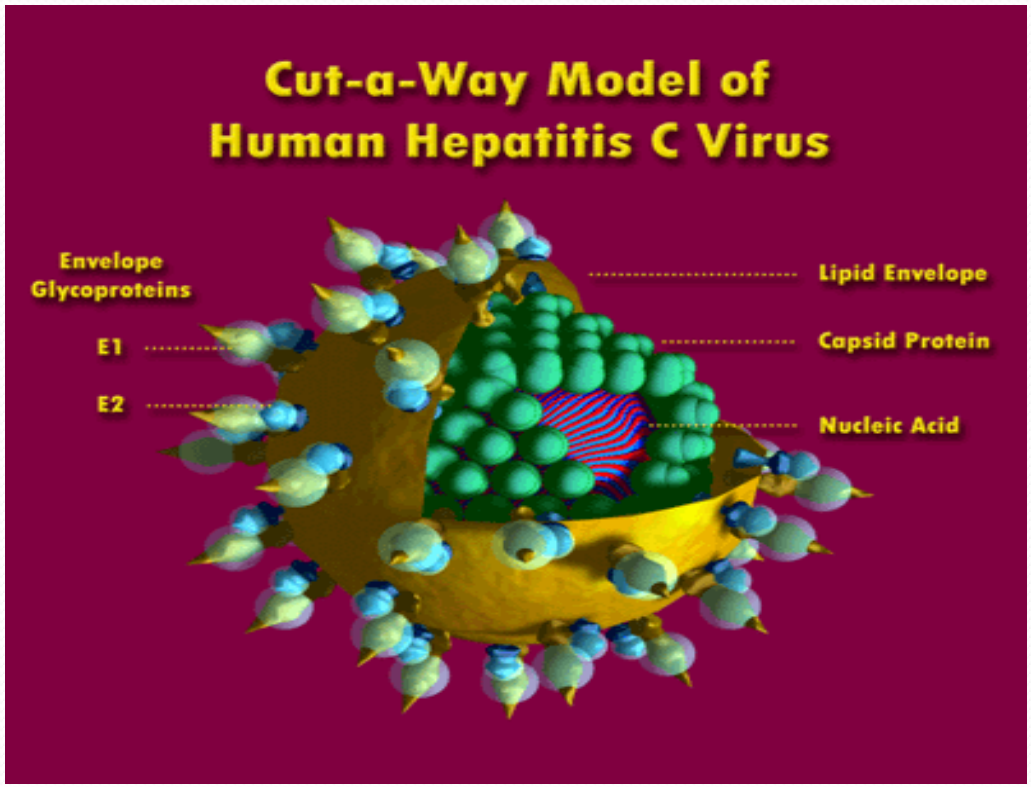
CDC

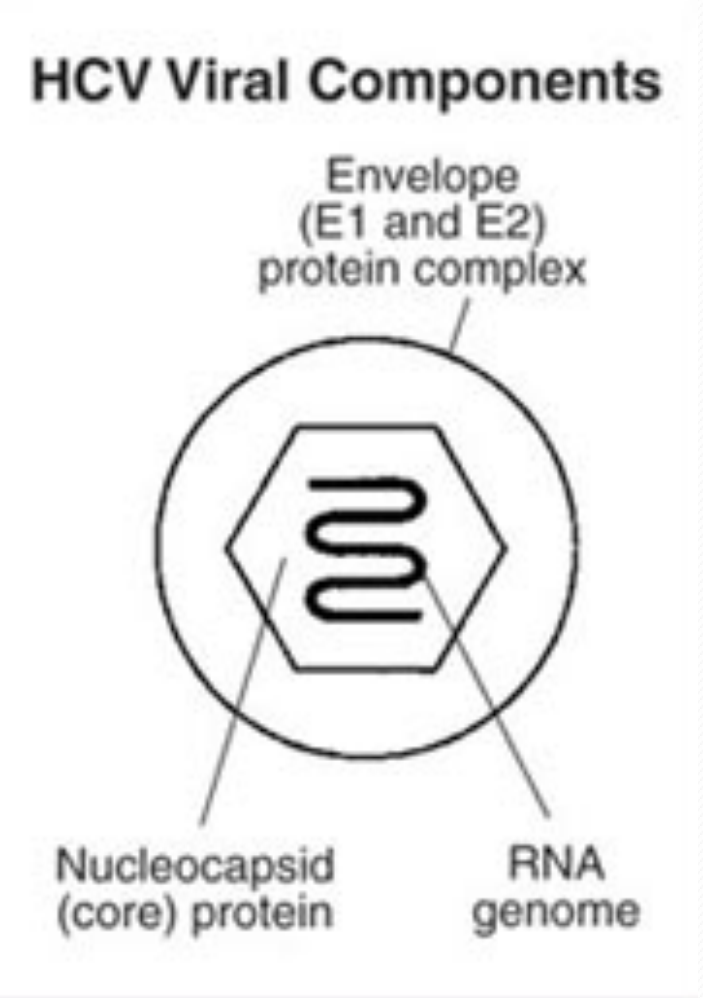
Natural History of HCV Infection



Alter, MJ. Epidemiology of Hepatitis C in the West. Semin Liver Dis. 1996: 15 5-14.
Management of Hepatitis C: NH Consensus Statement. 1997 Martin 24-26, 15(3)

The Hepatitis C Virus





HCV is a small (40 to 60 nm in diameter), **enveloped, single-stranded RNA virus .**

The Hepatitis C Virus

- **the virus mutates rapidly, changes in the envelope proteins** (help it evade the immune system).

- **Six major genotypes and more than 50 subtypes of HCV.**

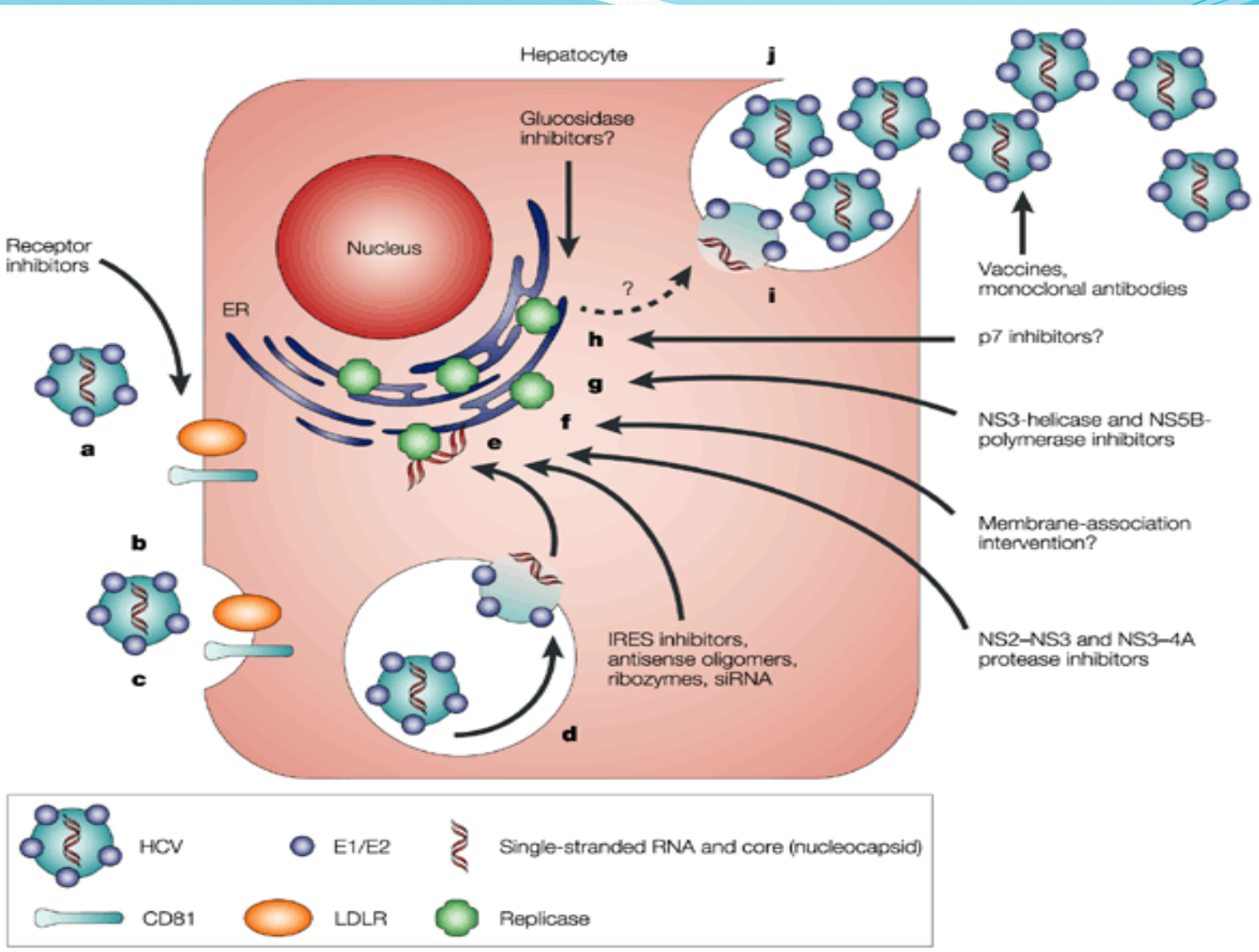
- **The different genotypes have different geographic distributions.**

Genotypes 1a & 1b are the most common in the U.S. (about 75 % of cases).

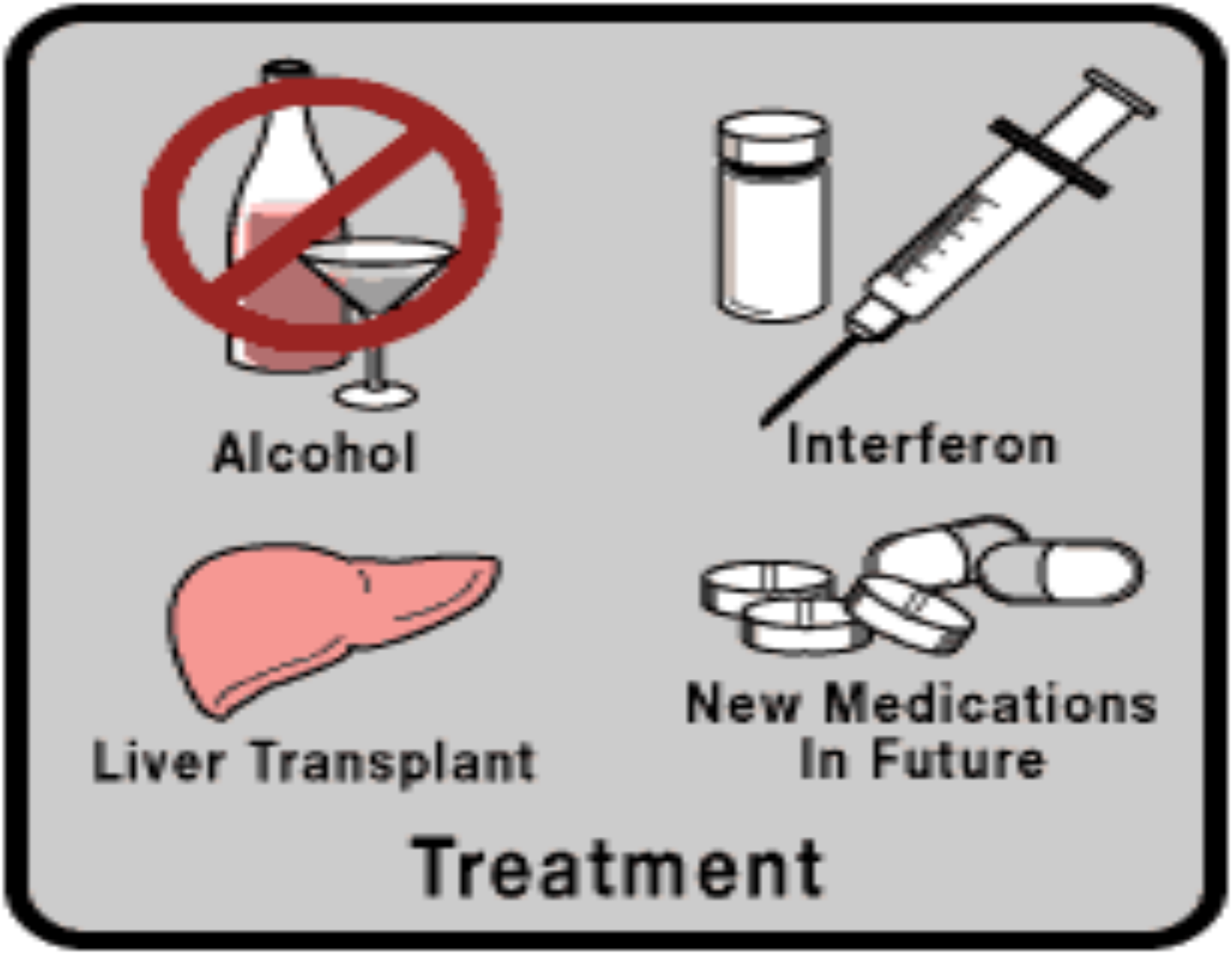
Genotypes 2 & 3 (present in only 10 to 20 % of patients).

- There is **little difference in the severity of disease** or outcome of patients infected **with different genotypes.**

However, **Patients with genotypes 2 & 3** are more likely **to respond** to **interferon treatment.**



Nature Reviews | Drug Discovery


Alcohol
Interferon
Liver Transplant
New Medications
In Future
Treatment

Interferon-alpha & HCV

Is the **basis of treatment regimens** since the identification of HCV, either alone or in conjunction with the nucleoside analogue **ribavirin**.

The recent introduction of **pegylated forms of interferon-alpha**, with

- greater stability and in vivo activity,

- has substantially - improved **sustained virological response (SVR)** rates compared with unmodified interferon-alpha,

- with SVR rates of 35-66% when used in conjunction with ribavirin in randomized controlled trials.

Two **pegylated interferon (peginterferon)-alpha molecules** are commercially available for the **treatment of chronic HCV**,

these differ in the **size and nature of the covalently attached polyethylene glycol (PEG) moiety**,

with resulting differences in **pharmacokinetics & in dosing regimens**.

Peginterferon-alpha-2b

- has a **linear 12 kDa PEG chain** covalently attached primarily to
- histidine-34 of interferon-alpha-2b
- via an unstable urethane bond that is
- subject to hydrolysis once injected,
- releasing native interferon-alpha-2b

- a shorter half-life in serum than peginterferon-alpha-2a and
- requires bodyweight-based dosing.

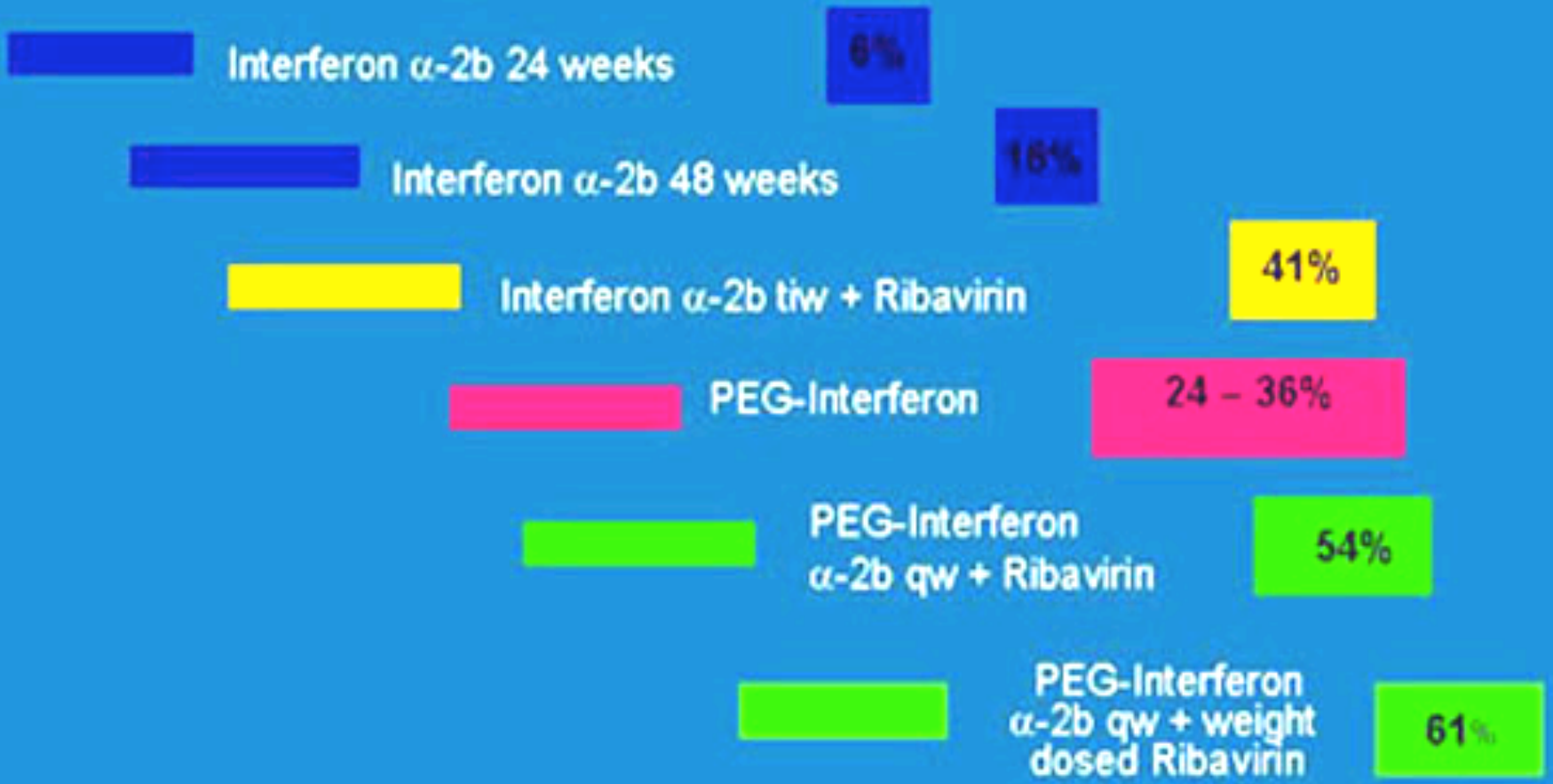
peginterferon-alpha-2a

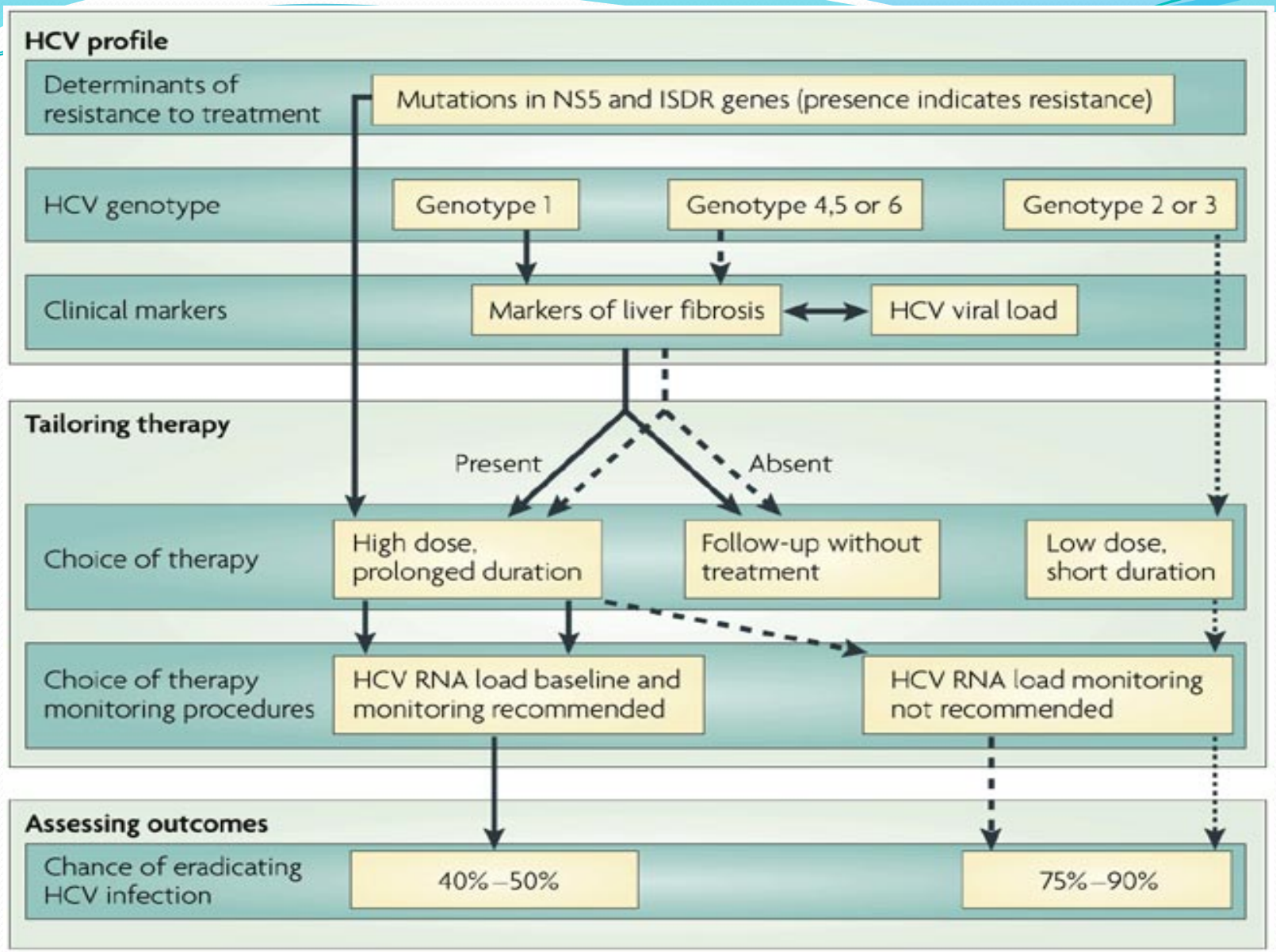
- has branched, 40 kDa PEG chain is covalently
- attached to lysine residues of interferon-alpha-2a,
- via stable amide bonds &
- circulates as an intact molecule.

- longer half-life and
- reduced clearance compared with native interferon-alpha-2a,
- can be given once weekly independently of body weight.

Peginterferon plus ribavirin, as the standard of care for patients with chronic hepatitis C

The Evolution Efficacy With Interferon Based Therapy Over the Last 10 Years





Nature Reviews | Microbiology

Hope through Research

Basic Research

A major focus of hepatitis C research has been to

* **Develop a tissue culture system that will enable researchers to study HCV outside the human body.**

- This goal was achieved in part in 2005 when three different laboratories reported tissue culture systems using HCV, genotype 2.

- These **systems are now being improved** and used to study **how the virus infects cells** and whether **spread can be blocked by antibodies and by different antiviral d**rugs.

* **Animal models and molecular approaches to the study of HCV** are also important.

* **Understanding how the virus replicates and how it injures cells** would be helpful in developing a means of controlling it and in screening for new drugs that would block it.

Hope through Research

New Treatments

Most critical for the future is

the development of new antiviral agents for hepatitis C.

Most interesting will be:

- Specific inhibitors of HCV-derived enzymes

such as protease, helicase, and polymerase inhibitors.

- Drugs that inhibit other steps in HCV replication by :

* **blocking production of HCV antigens from the RNA** (IRES inhibitors),
* **preventing the normal processing of HCV proteins** (inhibitors of glycosylation),
* **blocking entry of HCV into cells** (**by blocking its receptors**).

* **blocking the cell injury caused by the virus infection.**

(by using nonspecific cytoprotective agents)

Hope through Research

New Treatments

Further, **Molecular approaches to treat hepatitis C are worthy of investigation;**

(using **ribozymes,** which are enzymes that break down specific viral RNA molecules, and inhibit viral replication).

Hope through Research

Diagnostic Tests

More sensitive and less expensive assays for measuring HCV RNA and antigens in the blood and liver are needed.

- **Convenient tests to measure HCV in serum and to detect HCV antigens in liver tissue would be helpful.**

- **Clinically, noninvasive tests such as ultrasound elastrography that would reliably *predict liver fibrosis* would be a very valuable advance.**

Hope through Research

Prevention

At present, the only means of preventing new cases of hepatitis C are to

- **Screen the blood supply,**
- **Encourage health professionals to take precautions when handling blood and body fluids**
- **Inform people about high-risk behaviors.**
- **All drug users should receive instruction in safer injection techniques**

Vaccines and immunoglobulin products do not exist for hepatitis C, development in the near future is not applicable because these products would require antibodies to all the genotypes and variants of hepatitis C.

Nevertheless, advances in immunology and innovative approaches to immunization may develop some form of vaccine for hepatitis C.

Screening of Plants for Anti-HCV

Selection of Plants for Anti-HCV activity based on:

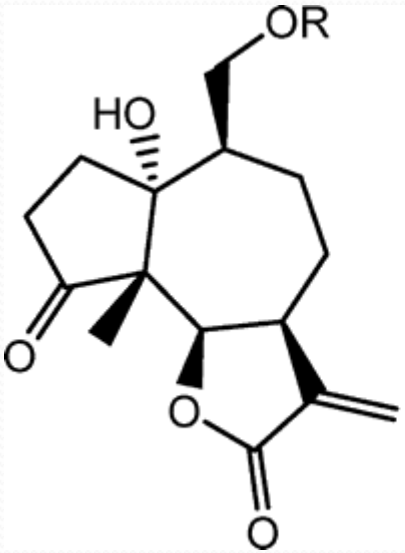
1 – Drugs used **in Folk medicine**

2 – Previous screening of **several Egyptian plants for anti-HIV activity.**

3 – Search for plants, **having molecules with similar active moiety**

Anti-HCV Bioactivity of Pseudoguaianolides from *Parthenium hispidum*

(**Hu *et al. 2007***)
Compounds 2−4 were found to possess **in vitro anti-HCV activity** in the subgenomic HCV replicon system



1: R = *n*-propanoyl
2: R = *n*-butanoyl
3: R = tigloyl
4: R = 3-methyl-2-butenoyl
5: R = angeloyl
6: R = acetyl
7: R = isopropanoyl
8: R = 2-methylbutanoyl
9: R = 3-methylbutanoyl

The antiviral activities of *Anethum graveolens* and *Foeniculum vulgare*

- **Abd El-Baset *et al.* 2001**
- for the treatment of **cancer and viral diseases**

Proanthocyanidin from Blueberry Leaves as Anti-HCV

- Takeshita ***et al.*** *2009*

- **Proanthocyanidin from Blueberry Leaves Suppresses Expression of Subgenomic Hepatitis C Virus RNA*.**

Licorice as Anti-viral drug

- **Bouras *et al.* 2001**

  Their studies have revealed that the healing properties of licorice components could be effective against a much wider spectrum of diseases, such as

  chronic hepatitis and **HIV infection.**

GL has been widely used in Japan for the **treatment of chronic hepatitis B and C** (CHC-CHB) in patients with severe side effects from interferon.

Anti-HCV Activities of Lignan Extracted from Flaxseed

- **(Barbary *et al.* 2010)**
- In-vitro anti-HCV screening of **flaxseed lignans** revealed significant effect on RNA replication with concentration at **100 µg /ml.**
- However, the lignan extracts showed negative results when used at Conc. at **5, 25, and 50 µg/ml.**

Tropolone derivatives as anti-HCV therapy

- **Chachulska *et al*. 2006**
- these synthetic compounds could be further modified to develop **potent inhibitors of the HCV helicase** and of **viral replication**.

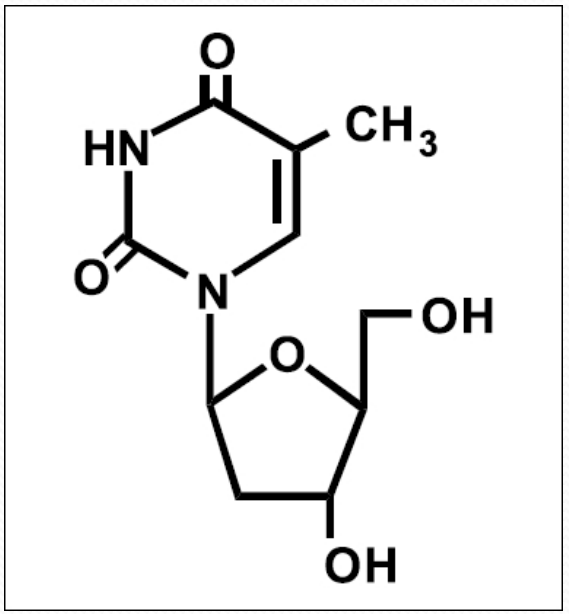
Telbivudine, (L-deoxythymidine) **as Anti-HBV**

- **(Fung *et al.* 2008)**
- **Telbivudine** , (L-deoxythymidine)

a new treatment option in

the management of

chronic Hepatitis B



Gypsophin: A novel a-glucosidase inhibitory

- **Luo *et al. 2007)***

  cyclic peptide, with a pyrrolidine-2,5-dione unit from the roots of ***Gypsophila oldhamiana***

The serious nature and the frequency of hepatitis C in the population

make

the search for new therapies of prime importance.

Thank You