Ghazala Rubi

B.Sc, M.Sc,

F.I.B.M.S;

MPhil (Mol.Bio)

PhD (Human Genetics & Mol.Bio)

Personal Information

- **Personal Detail**
  - **Name** : **Ghazala Rubi**
- **email** **rubighazala@yahoo.com**
- Nationality : Pakistani
- Place of Birth : Lahore, Pakistan
- Marital Status : Married

Profession

- **FULL-TIME EMPLOYMENT**
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- 1995- 1997 **Clinical Microbiologist** at Shaukat Khanum, Memorial Cancer Hospital and Research Centre Lahore, Pakistan.
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- 1997-2008 Clinical Senior **Molecular Biologist (head of the PCR diagnosis**) at Chughtais Lahore Lab, Ammar Medical Complex, 8-Jail Road, Lahore. Pakistan.
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- 2008-2013 **Senior teaching & Research faculty** at University of Health sciences Lahore.
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- 2013-todate **Molcular Pathologist** at Agha Khan university Hospital, Pakistan
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HONOURS

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- Got an award for presenting a paper in a conference for an outstanding research **AWARD** at *International Conference of Hepatology in Avari Hotel Lahore 30-31st Jan, 2011.*
- Academic Roll of Honor from Government College, Lahore.
- Elected Secretary of Biological Society of Government College, Lahore.
- Fellow of Institute of Biomedical Sciences of UK (FIBMS)
- Visiting teacher at Institute of Biotechnology and Biochemistry, University of the Punjab, Lahore Pakistan.
- Award for a scholar at the University of Health Sciences Lahore Pakistan.
- Active presenter at CME (continued Medical education) in Pakistan.
- Achieved 3rd prize in paper presentation in 37th Annual conference of PAP, Dec, 2013.
- Won an excellent award in paper presentation in 12th international congress on Ageing in Hyderabad India, 2014.
- Abstract accepted for an international conference in MEMBS, Dubai, sept,2014.

Training & Courses

- 15 Day course on Malariology and Control NIMRT Lahore (November 1992).
- Course on Molecular Genetics, Molecular Biology of Prokaryotes, Eukaryotes and plant, NCEMB from February 13-July 31, 1993.
- Computer Programs of DOS, Lotus 123, Word 5, Word 5.5, WinWord, FoxPro etc., NCEMB (February 15-April, 1993).
- PCR for clinical diagnosis, School of Biological Sciences, University of Surrey, UK. (November 96-May, 1997).
- Clinical investigations for bacterial and viral infections. Royal Free Hospital, London, UK (April 1997).
- Molecular development course (Jan-Mar 1997) School of Biological Sciences University of Surrey, UK.
- Molecular genetics course (Jan-Mar 1997) School of Biological Sciences University of Surrey, UK.
- Molecular biological course on gene sequencing (25th Sept to 30th Sept 2000) held at Agha Khan University, Hospital Karachi, Pakistan.
- Training on HLA tissue typing in transplant patients (July 2003).
- Training on REAL TIME PCR for HCV and Genotyping patients in Italy from 9th May to 13th May 2005.
- 24 weeks 1st April 2010-30th Sept 2010, course on Molecular Biology at University of Health Sciences Lahore.
- National Course on Human Genetics 6th March, 2011-30th April 2010, at University of Health Sciences Lahore.
- Two months training/course in Pathology, virology, bacteriology, Genetics, chemical pathology and Molecular Pathology, and CAP quality control in pathology, 1st Jan – 28th February, 2013.

Research interests:

1) **Molecular Diagnostics**

- HCV qualitative & HCV Quantitative
- HCV Genotyping
- IL 28B GenePolymorphism
- HBV Qualitative & Quantitative
- HBV Genotyping
- MTB Diagnosis from all body fluids
- CMV Diagnostics
- Dengue Diagnosis

HLA Tissue Typing

- HLA tissue typing by PCR diagnosis for Transplant Purpose.
- HLA tissue typing for donor and recipient
- Matching of donors and recipients
- Description of results to patients and clinicians
- CME in hospitals for education purpose

HCV

- My special interest with HCV RNA and patients suffering with this virus.
- How did they acquire and sufferings?
- Treatment and viral load coordination
- Genotype description
- Help those patients to reach clinicians
- Kind of therapy and quality testing

Response to therapy

My research included

- data of different communities getting the HCV infection.
- Response of therapy
- Not responded to the therapy
- Realpsers after therapy

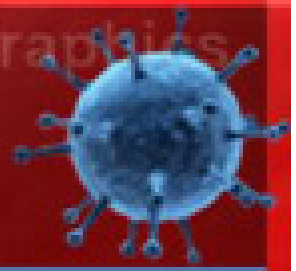
Ageing

- How we are corelating ageing with current infections
- Ageing of HCV RNA in different age groups
- How our ecology is effecting this process
- Written two grants on it

HCV and Diabetes

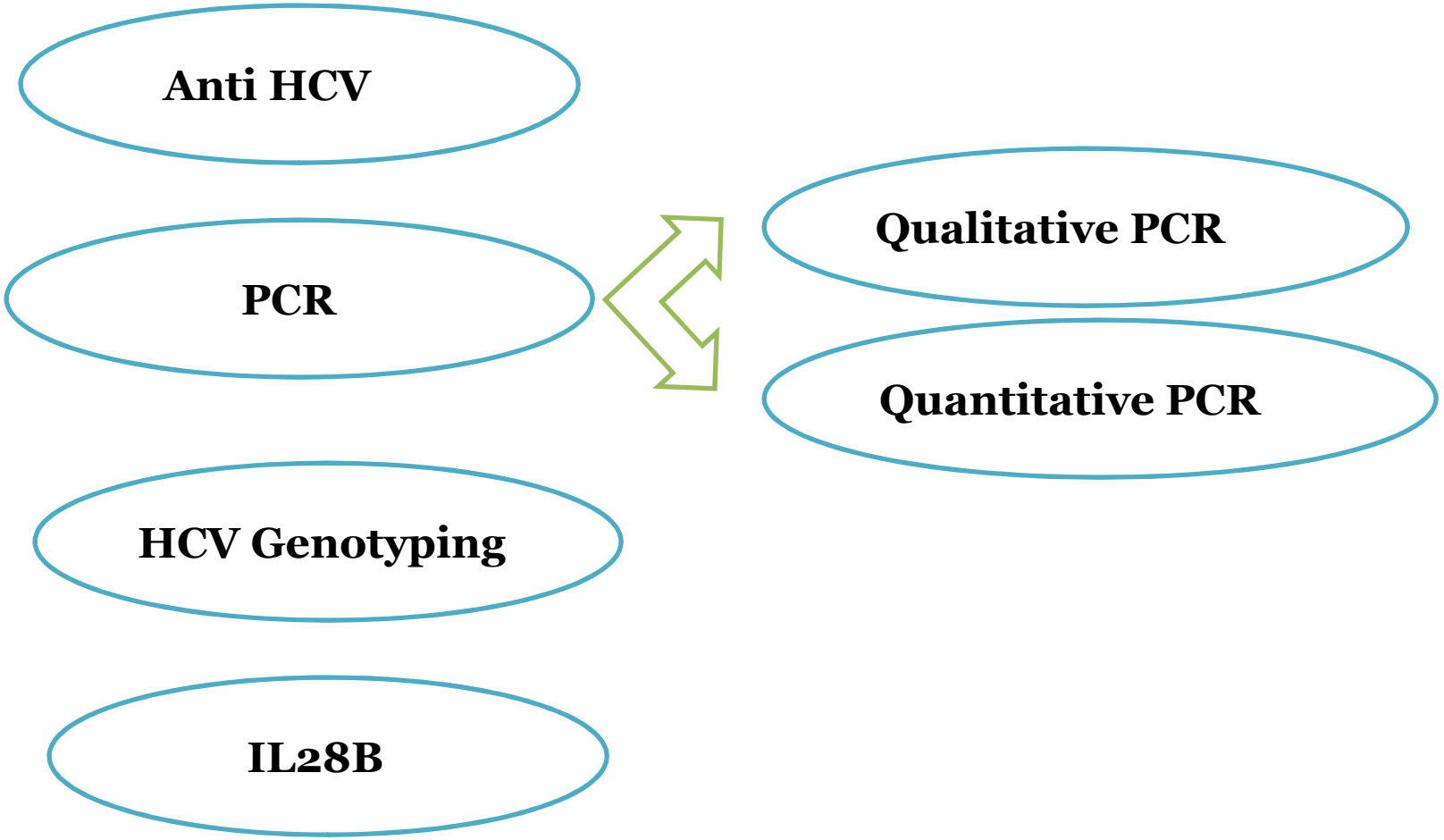
- Mostly patients of HCV acquired diabetes Mellitus after therapy
- Trying to get grant on it to work on these two projects

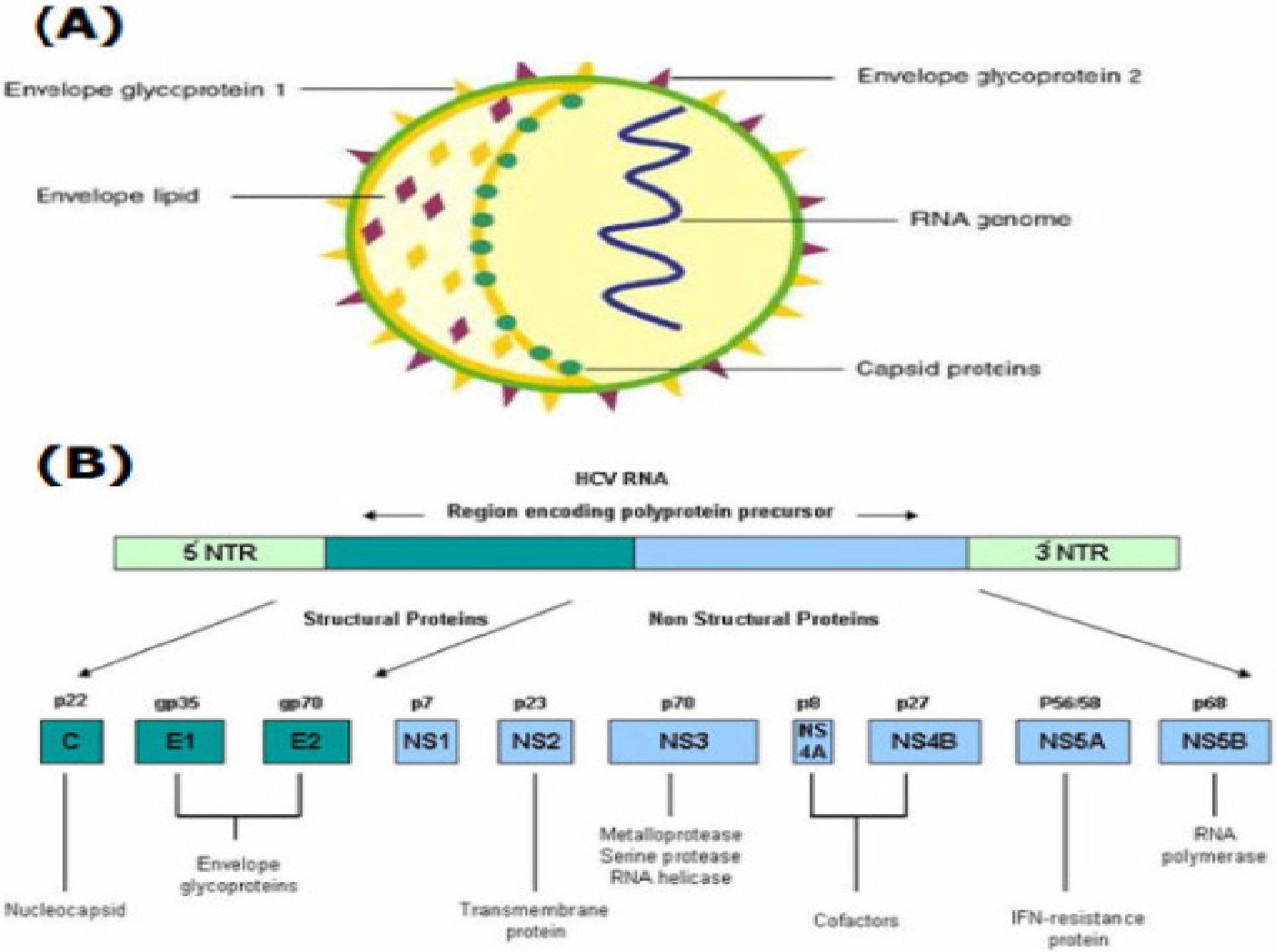
Viral Hepatitis
Role of Diagnostic Testing



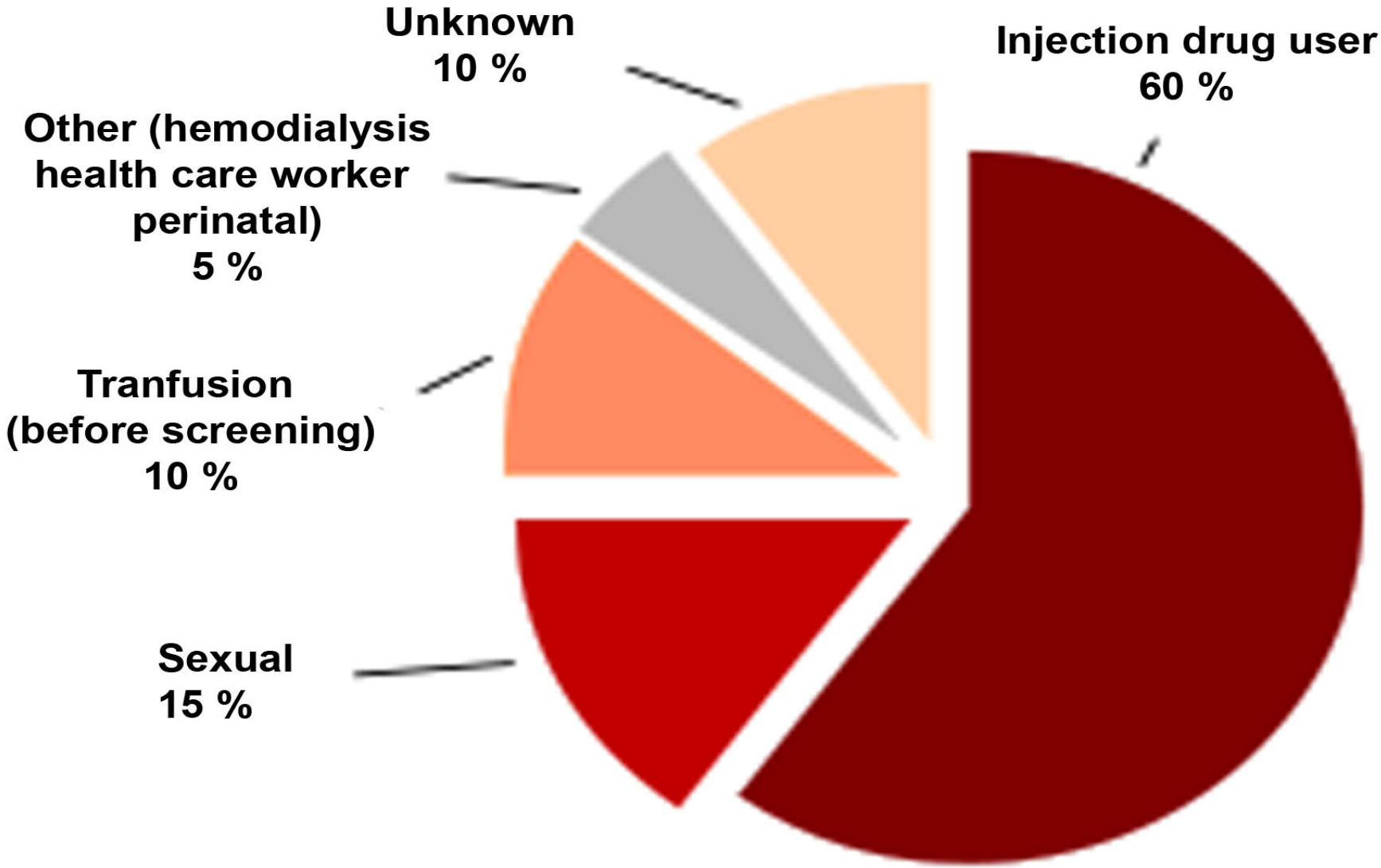
- **Identify patients with viral hepatitis infection**
  - Previous exposure to hepatitis virus
  - Active infection
  - Inactive infection
  - Resolved infection
- **Assess response to therapy**
  - Prior to onset of treatment
  - During and following treatment

What testing should be done?

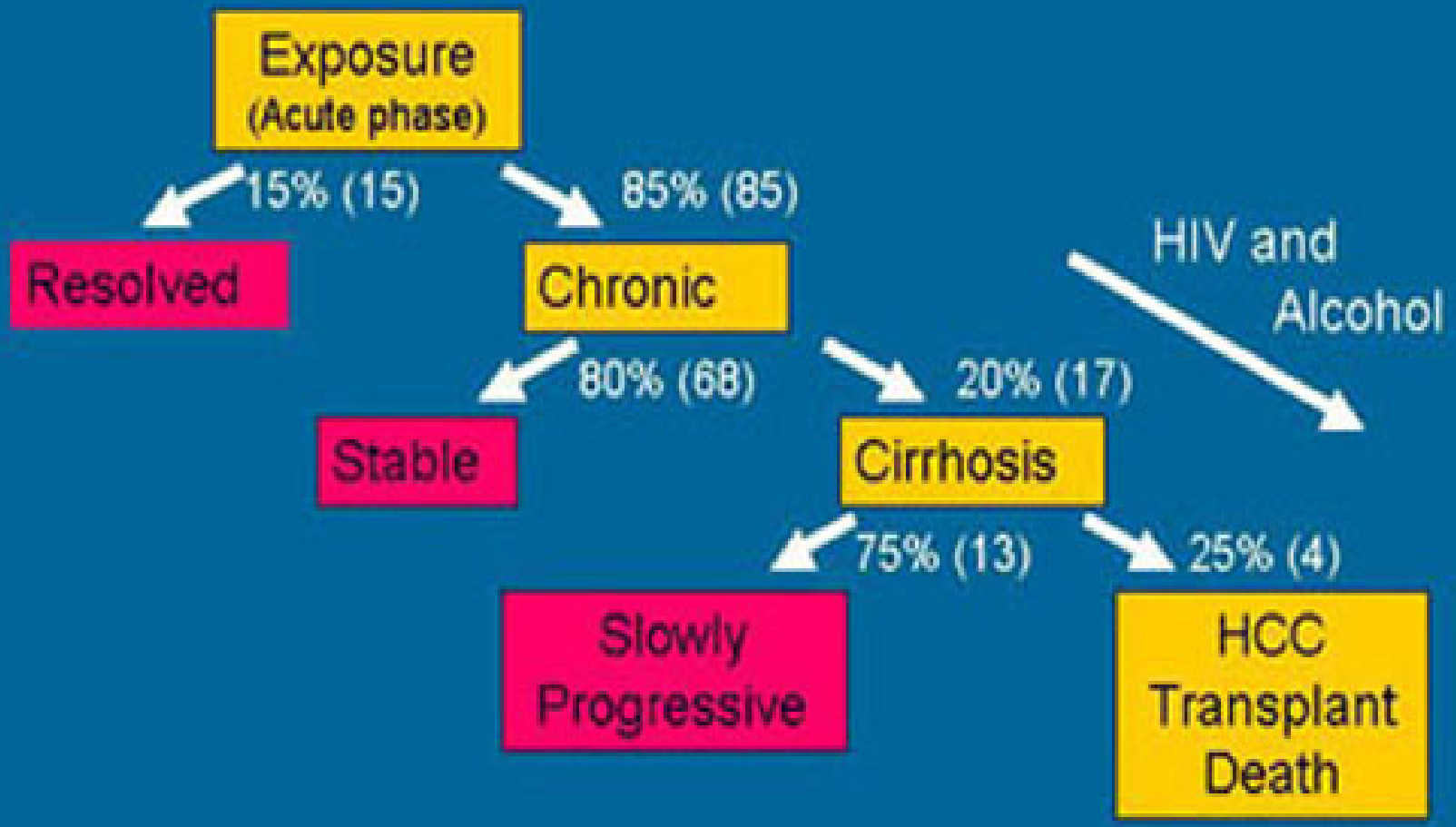



(A)
Envelope glyccprotein 1
Envelope glycoprotein 2
Envelope lipid
RNA genome
Capsid proteins
(B)
HCV RNA
Region encoding polyprotein precursor
5' NTR
3' NTR
Structural Proteins
Non Structural Proteins
p22
gp35
gp70
p7
p23
p70
p8
p27
P56/58
p68
C
E1
E2
NS1
NS2
NS3
NS 4A
NS4B
NS5A
NS5B
Nucleocapsid
Envelope glycoproteins
Transmembrane protein
Metalloprotease
Serine protease
RNA helicase
Cofactors
IFN-resistance protein
RNA polymerase

% age of HCV modes of infection

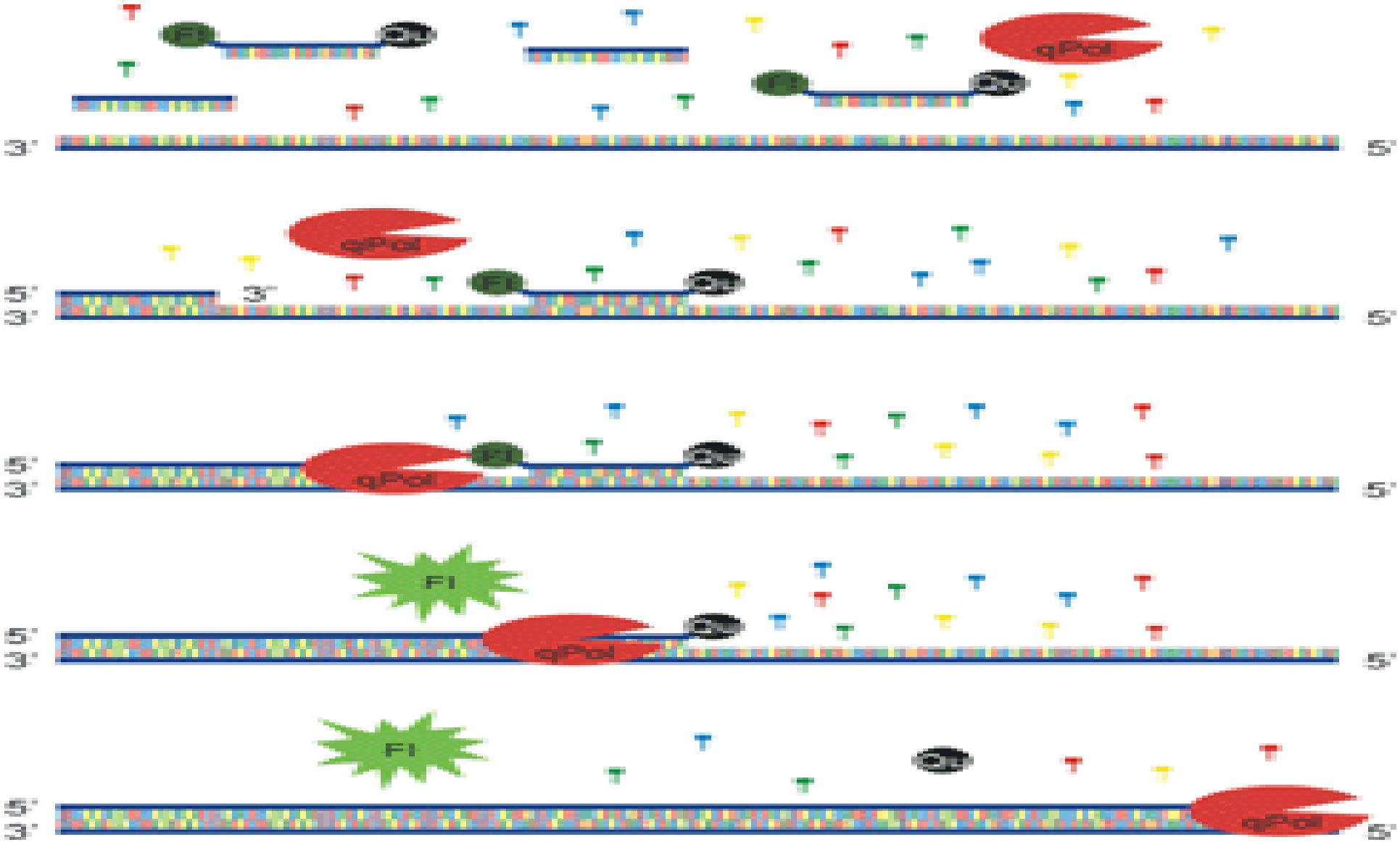


Natural History of HCV Infection



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

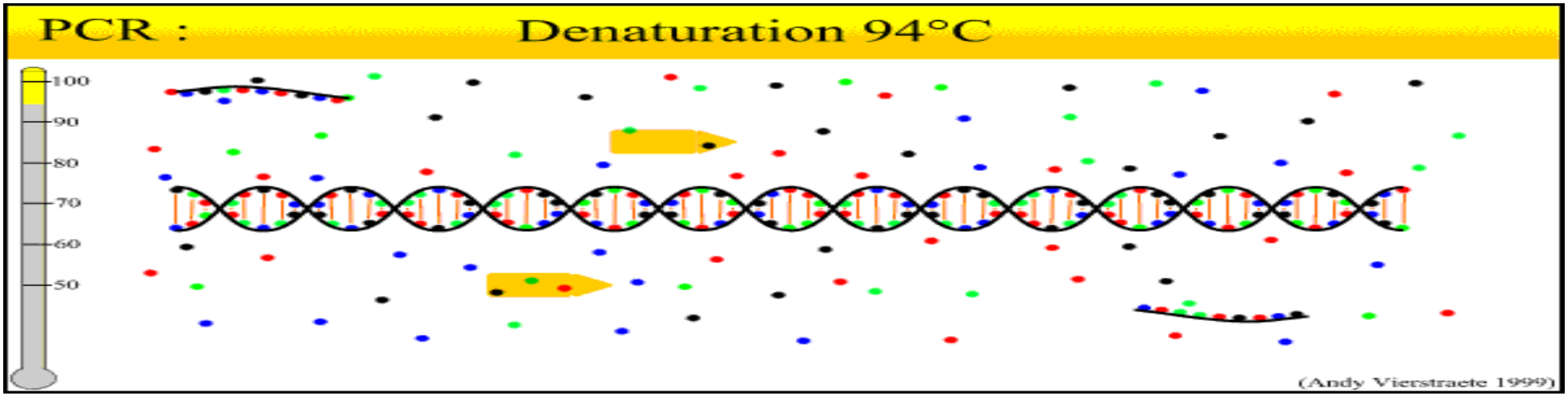
Real Time PCR

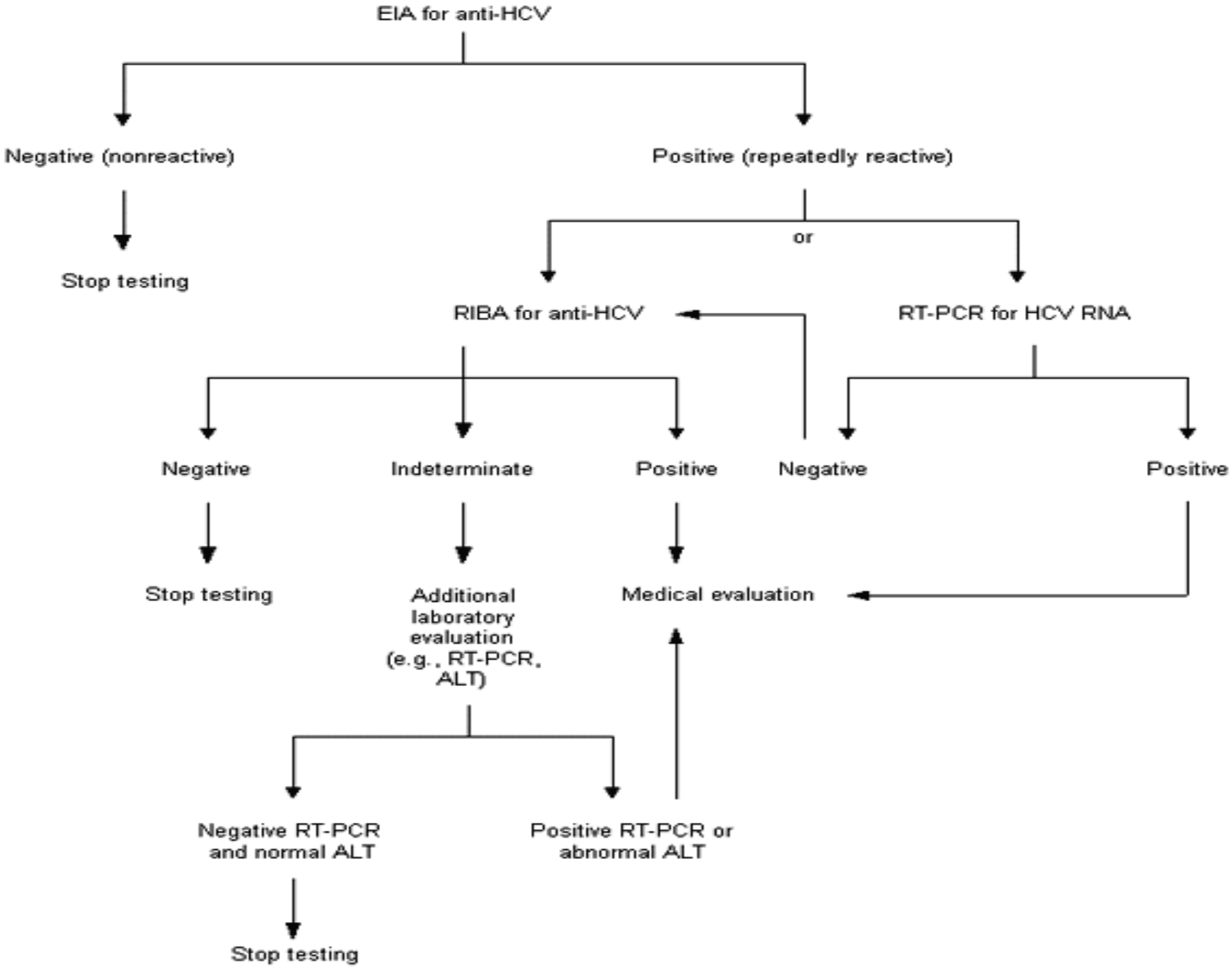


How does PCR work?

1. *Denaturation*
2. *Aneeling*
3. *Extention*

The cycle of denaturing and synthesizing new DNA is repeated as many as 30 or 40 times, leading to more than one billion exact copies of the original DNA segment.
It is directed by a machine called a thermocycler, which is programmed to alter the temperature of the reaction every few minutes to allow DNA denaturing and synthesis.




EIA for anti-HCV
Negative (nonreactive)
Stop testing
Positive (repeatedly reactive)
or
RIBA for anti-HCV
RT-PCR for HCV RNA
Negative
Indeterminate
Positive
Negative
Positive
Stop testing
Additional laboratory evaluation (e.g., RT-PCR, ALT)
Medical evaluation
Negative RT-PCR and normal ALT
Positive RT-PCR or abnormal ALT
Stop testing

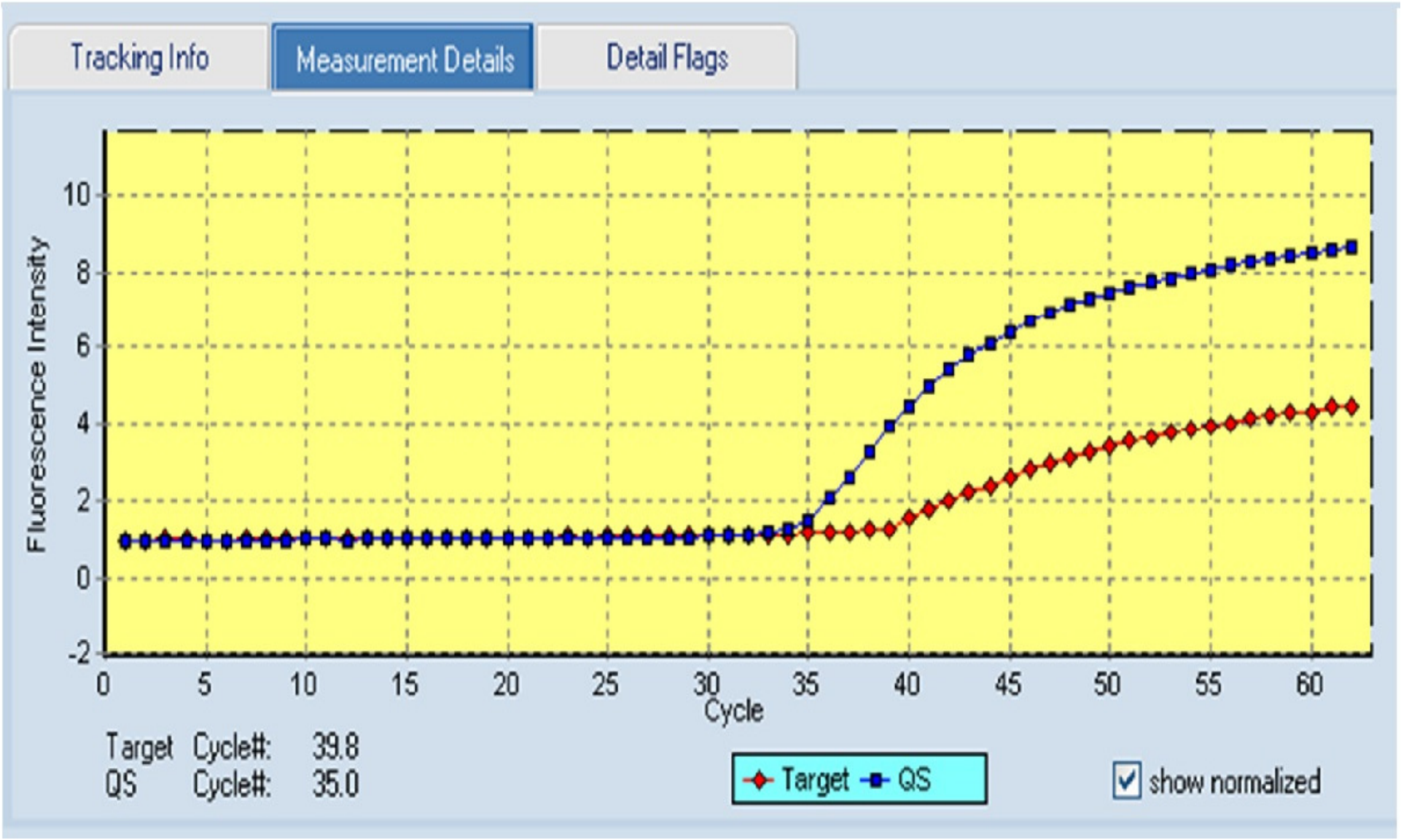
Ampliprep &TaqMan® System

Qualitative HCV PCR

3 Steps

- ***Extraction……(Ampliprep)***
- ***Reverse Transcription, cDNA……..(Taq Man 96)***
- ***Amplification & Detection……(Taq Man 96)***

Results Interpretation by graph



Genotypes of HCV

- ***The ability of the virus to mutate has resulted in the existence of***

  11 different genetic variations of HCV.

- ***These variations are known as 'genotypes'.***

- ***Numbered from 1 - 12.***

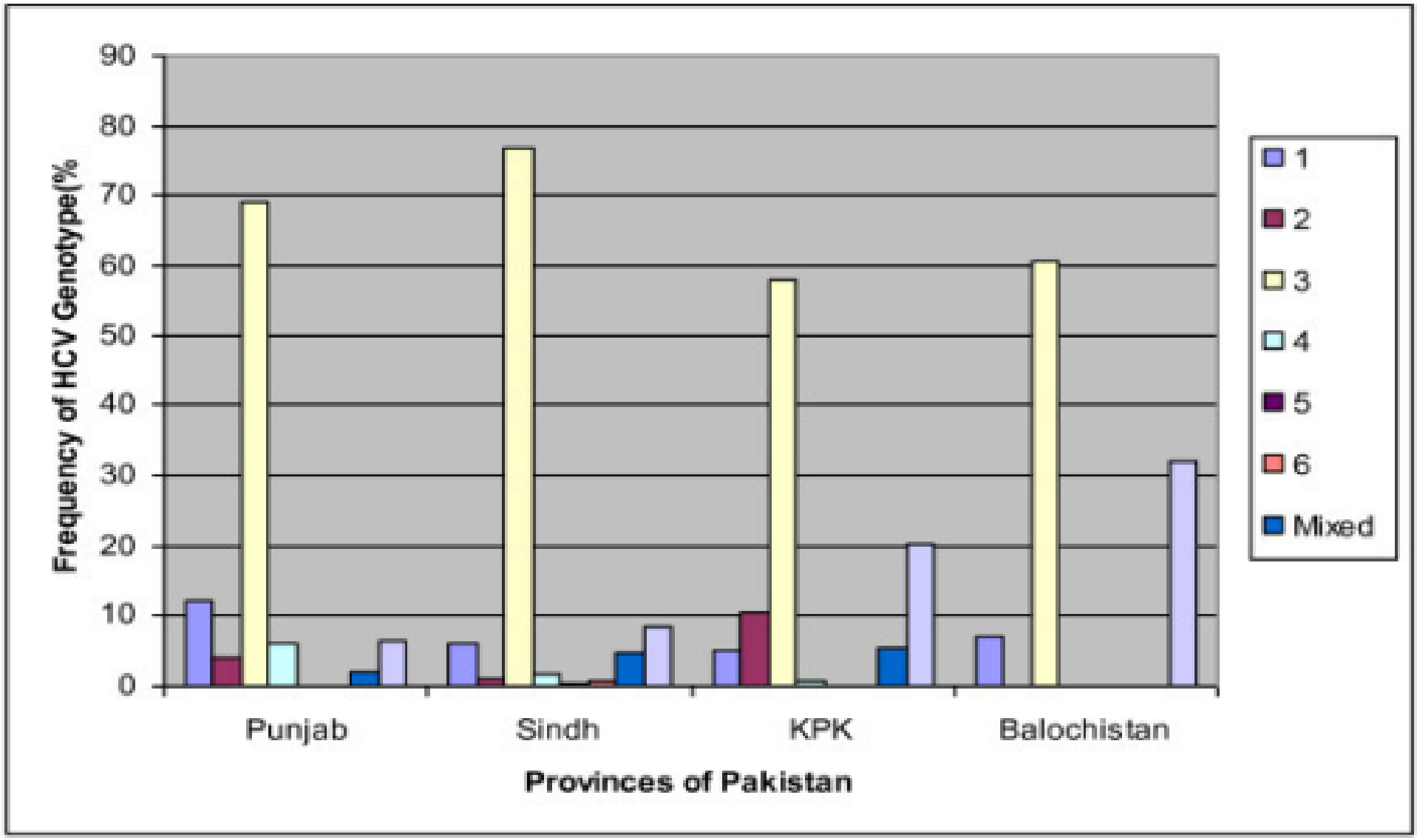
- ***These genotypes also have sub-types.***

- ***The different genotypes are often, but not exclusively, related to different parts of the world.***

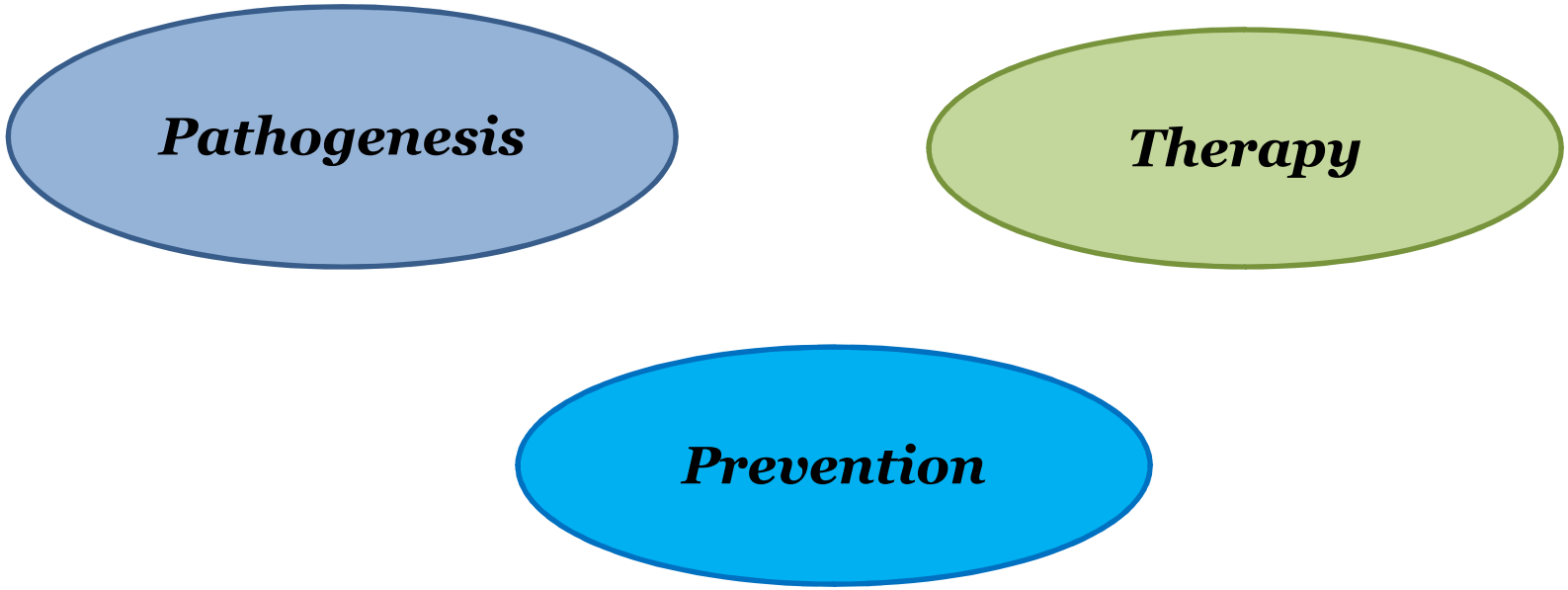
Genotype distribution

- **Genotypes 1, 2 and 3 have a worldwide distribution.**
- **Types 1a and 1b are the most common, accounting for about 60% of global infections.**
- **They predominate in Northern Europe and North America and in Southern and Eastern Europe and Japan.**
- **Genotype 2 is less frequently represented than type 1.**
- **Genotype 3 is endemic in south-east Asia.**
- **Genotype 4 is principally found in the Middle East, Egypt, and central Africa.**
- **Type 5 is almost exclusively found in South Africa. Genotypes 6 in China.**

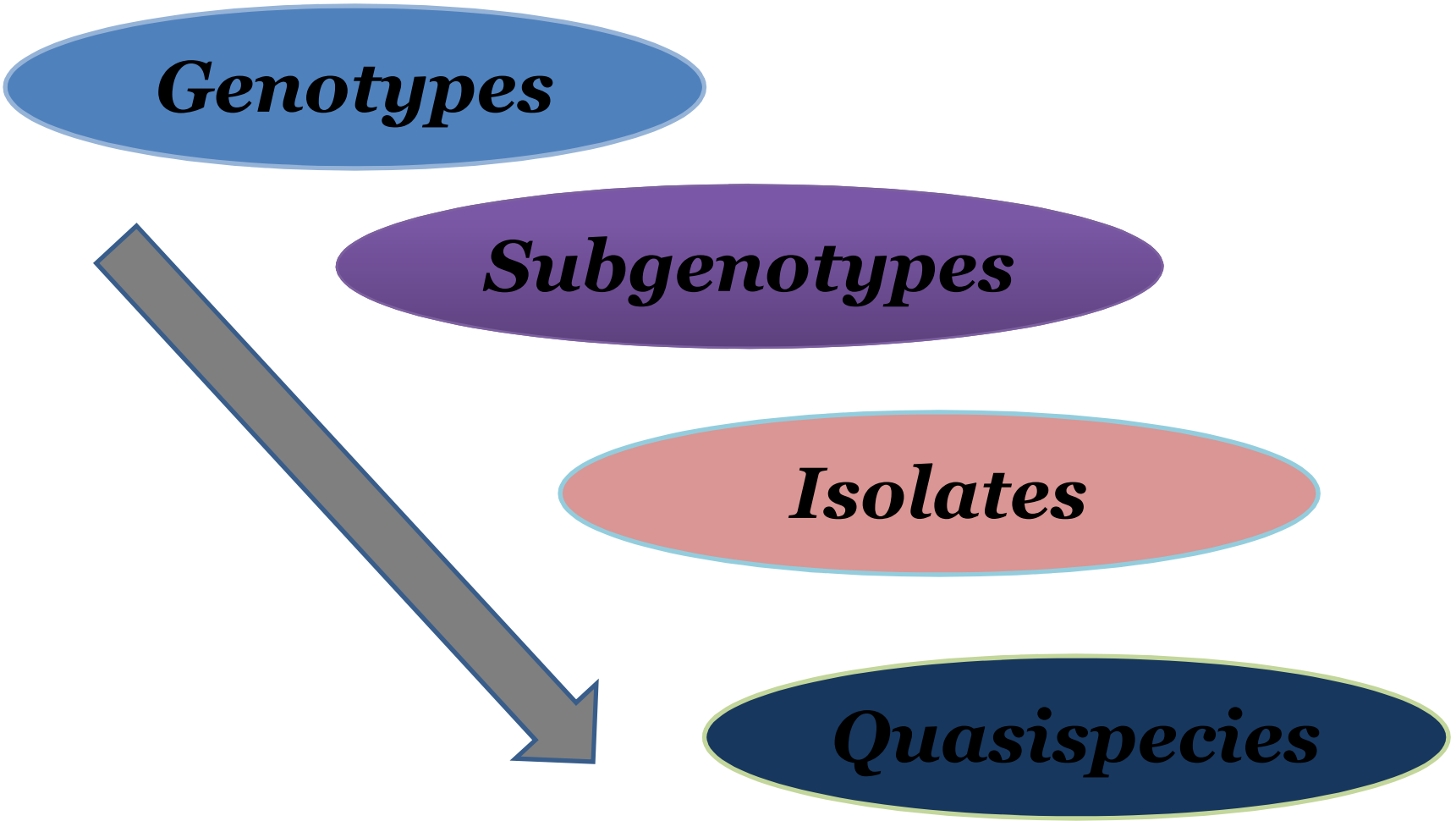
HCV Genotype distribution in Pakistan



Genetic Variability
Implications in HCV infection



Genetic Heterogeneity of Hepatitis C Virus



Genotype Assays

Different assays are used to determine genotype

Molecular Methods (Genotyping)

- ✓ ***Direct sequence analysis***
- ✓ ***Reverse Hybridization***
- ✓ ***RT PCR with genotype specific probes***

Serological Methods (Serotyping)

- ✓ ***Competitive ELISA***

- ➢ ***Most genotype assays use amplification of virus sequences by PCR.***
- ➢ ***Assays for determining genotypes and serotypes are***
- ➢ ***commonly employed in research settings***

IL 28B gene polymorphism

- ***IL28B (Interleukin 28B; interferon lambda3) genetic variations found on the human interferon gene on chromosome 19.***

- ***These variations may be a predictor regarding the effectiveness of interferon treatments for HCV.***

- ***There are 3 variations of the IL 28B genotypes:***

1. ***CC,***
2. ***CT***
3. ***TT.***

Patients with the CC allele have the best response to current treatments, people with CT and TT alleles have improved responses with the addition of the new DAAs. ERVR and achievement of virological milestones are still considered to be the best predictors for SVR.

It is important to remember…

- ***IL28B is a blood test***

- ***Just because you do not have the gene type does not mean you will not be cured of hepatitis C***

- ***some people who do not have this type can still be cured of hepatitis C***

- ***The CC genotype is different from what we usually call a genotype. HCV genotypes are strains of hepatitis C that are numbered 1, 2, 3, 4, 5 and 6 such as HCV genotype 1***

Possible treatment algorithms for chronic HCV genotype 1 infected patients according toIL28Balleles

HCV genotype 1 infection

rs12979860 CC

- pegIFN + riba
  24-48 weeks
- pegIFN + riba + TVR/BOC
  (12?)-24 weeks if eRVR
  48 weeks if no eRVR

rs12979860 CT/TT

- pegIFN + riba + TVR/BOC
  24 weeks if eRVR
  48 weeks if no eRVR
- pegIFN + riba (48-72 weeks)
  if contraindication to
  triple therapy
- quadrupel therapy?
- pegIFN + riba +
  NS5B/cyclophilin inhibitor?

Vote of credit

Challenge?

- Keen to do more research on Genetic Susceptibility of HCV RNA
- To do research on NS5B target region
- Why all patients could not get treated?
- Want to do cohort study from different regions of the world to establish a proper guide line for the patients and clinicians.

Agha Khan University Hospital