Laboratory and Clinical Diagnosis of HCV Infection

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I

Nonspecific Liver Tests

Biological Markers of Liver Disease

- **Serum ALT and AST activity :**
  - **Not specific,**
  - **No prognostic value,**
  - **Marker of the response to antiviral therapy.**
- **Serum bilirubin and alkaline phosphatase activity :**
  - **Marker of associated cholestasis.**
  - **No prognostic value.**
- **Serum γ-glutamyl transpeptidase activity :**
  - **Not specific.**

Liver Biopsy Examination

- **Necro-inflammatory activity :**
  - Reflects the degree of necrosis and inflammation,
  - Important predictor of liver disease outcome,
  - Important to assess the indication for antiviral therapy in certain instances.
- **Fibrosis :**
  - Major prognostic significance.

Screening for Hepatocellular Carcinoma

- α-fetoprotein levels.
- Regular ultrasonographic evaluations (every 6 months ?).
- Confirmation :
  - Computerized Tomography (CT),
  - Spiral CT,
  - Magnetic Resonance Imaging,
  - Lipiodol CT,
  - Hepatic angiography.

II

HCV Virological Tests

Anti-HCV Antibody Detection

- **ELISA tests :**
  - **Very sensitive in immunocompetent patients,**
  - **Sensitive in hemodialysis and immunodepressed patients (except profound immunodepression),**
  - **Specificity : 99%.**

- **“Immunoblot“ tests :**
  - **No utility in the diagnostic context.**

Qualitative HCV RNA Detection

- "Polymerase chain reaction" (PCR)
  - Amplicor HCV v2.0 (Roche),
  - Lower limit of detection : **50 IU/ml.**

- "Transcription-mediated amplification" (TMA)
  - Versant HCV RNA Qualitative Assay (Bayer),
  - Lower limit of detection : **10 IU/ml.**

HCV RNA Quantification

- “Polymerase chain reaction“ (PCR)
  - (Cobas) Amplicor HCV Monitor v2.0 (Roche),
  - Range of quantification : **600 to 500,000 IU/ml.**
- “branched DNA“ signal amplification
  - Versant HCV RNA Quantitative Assay (Bayer),
  - Range of quantification : **615 to 7,700,000 IU/ml.**
- Other tests : SuperQuant (NGI), LCx (Abbott), real-time PCR…

Molecular Determination of HCV Genotype ("Genotyping")

- **Direct sequencing :**
  - **Home-made : NS5B, E1 regions,**
  - **5 ' noncoding region (Trugene HCV 5'NC Genotyping Kit, Visible Genetics).**
- **Reverse hybridization :**
  - **INNO-LiPA HCV II (Innogenetics).**

***NB** : . **Typing** errors in 5' NC : exceptional.*

*. **Subtyping** errors in 5 'NC : 10%-25%, without clinical incidence.*

Serological Determination of HCV Genotype

- **Serological determination of HCV genotype (“serotyping“) :**
  - **Identifies the 6 types, but not the subtypes,**
  - **Interpretable in approximately 90% of patients with chronic hepatitis C,**
  - **Concordance with molecular tests : 95%.**

III

Practical Use of HCV Virological Tests

Diagnosis of Acute Hepatitis C

Anti-HCV Ab	HCV RNA*	Diagnosis
-	-	Not acute hepatitis C
-	+	Acute hepatitis C
+	-	Not acute hepatitis C
+	+	Hard to say...

**HCV RNA assay with LLD ≤ 50 IU/ml*

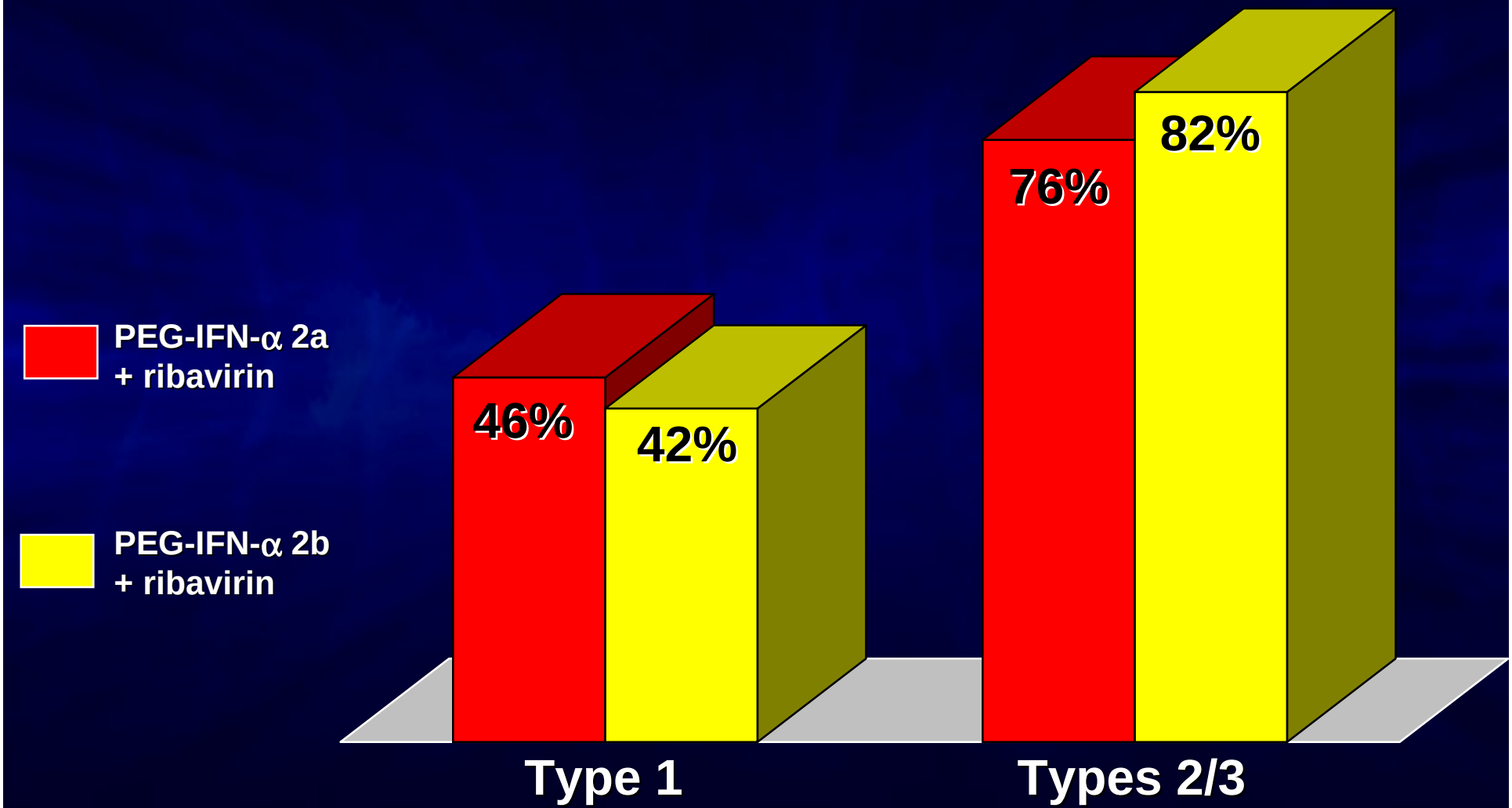
Diagnosis of Chronic Hepatitis C

- ELISA detection of anti-HCV antibodies,
- Confirmation by positive HCV RNA detection* if ELISA (+),
- HCV RNA detection* if ELISA (-) in hemodialysis and immunodepressed patients.

**HCV RNA assay with LLD ≤ 50 IU/ml*

PEG-IFN-α QW + ribavirin QD

Naive Patients (48 weeks)



(Manns et al., Lancet 2001;358:958-65 ; Fried et al., submitted)

Summary

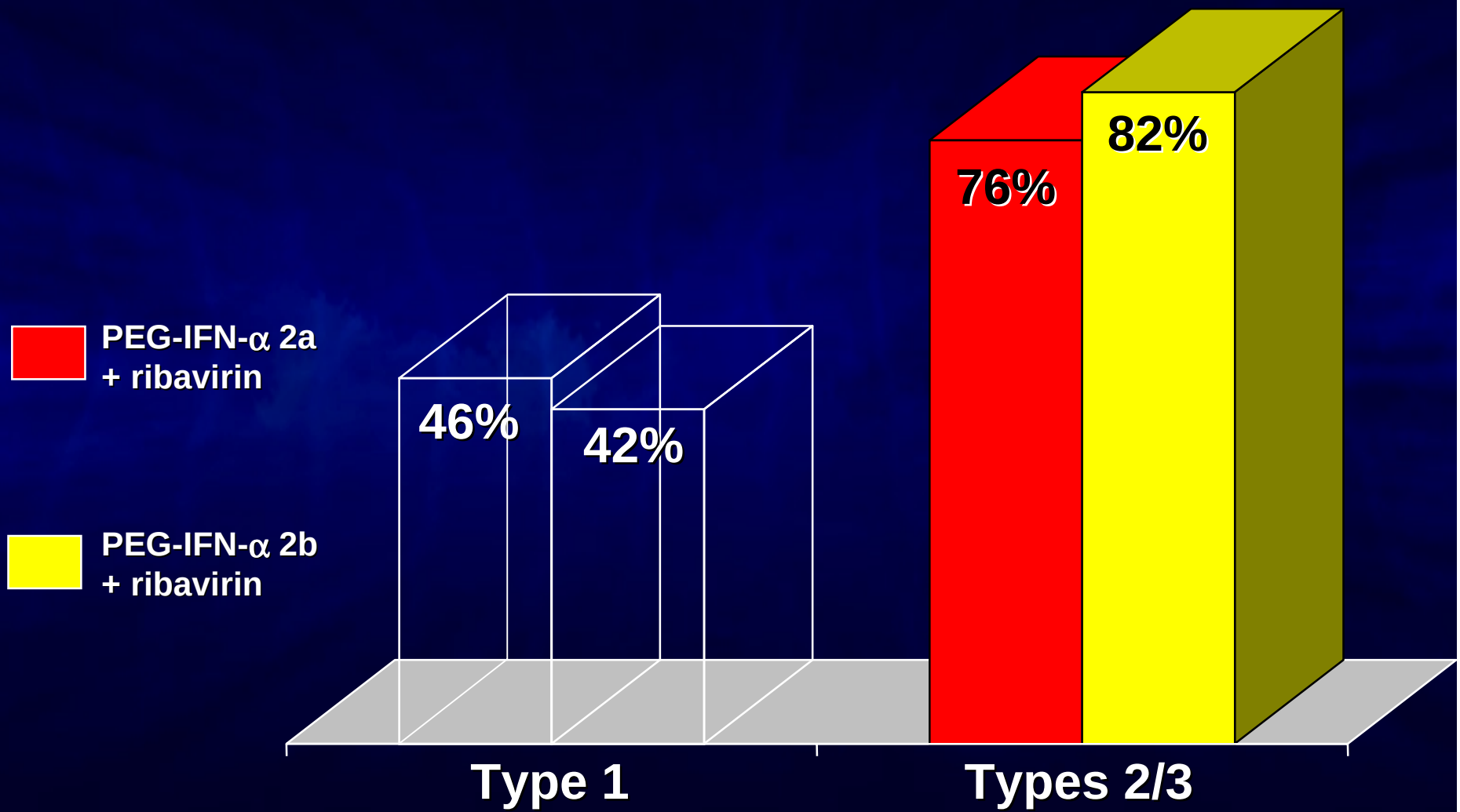
- **The outcome of antiviral therapy significantly varies according to the HCV genotype (genotype 1 vs genotypes 2/3).**

=> The therapeutic strategy will be different for genotype 1 and genotypes 2/3, respectively.

=> HCV genotype determination must be performed after the diagnosis of chronic hepatitis C is made if antiviral therapy is envisaged .

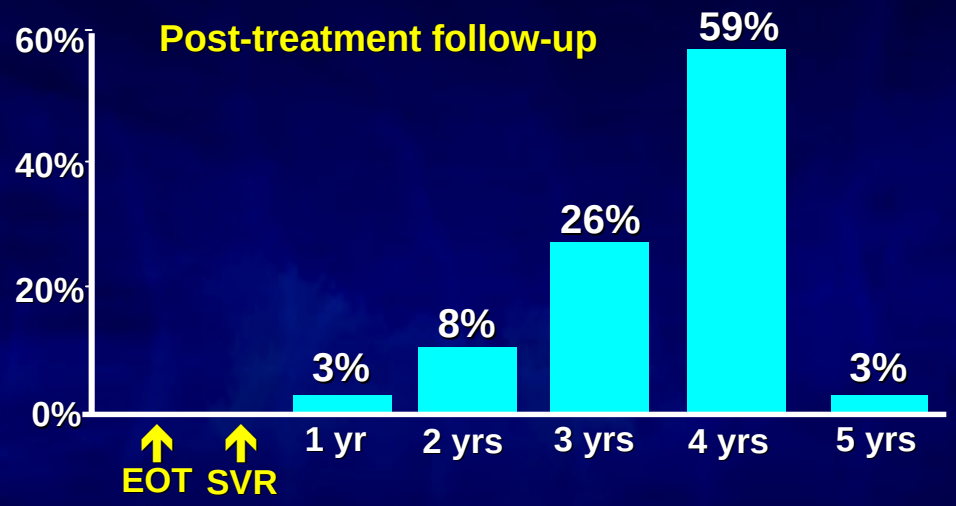
PEG-IFN-α QW + ribavirin QD

Naive Patients (48 weeks)



(Manns et al., Lancet 2001;358:958-65 ; Fried et al., submitted)

“Sustained Virological Response“



- *N = 395*

- **Persistence of response (RNA -) : 385/395 (97.5%),**
- **10 “late” relapses, all before 2 years.**

(McHutchison et al., AASLD 2001)

Summary

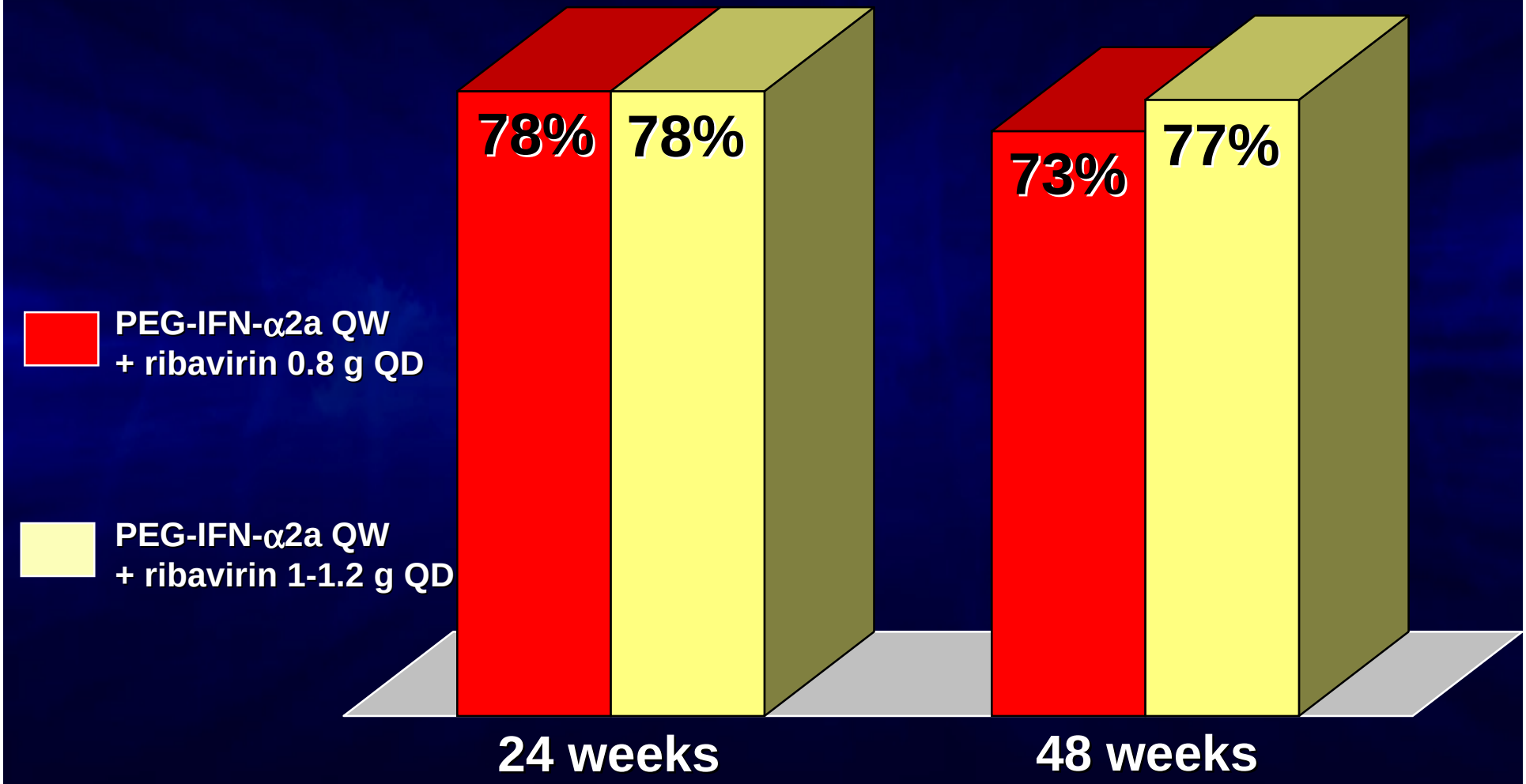
- Approximately 80% of patients infected with HCV genotypes 2 and 3 treated with PEG-IFN-α plus ribavirin achieve a sustained virological response.

- The sustained virological response appears to be associated with a definitive cure of infection in the vast majority of cases.

=> It is reasonable to propose antiviral treatment to all of the patients with detectable HCV RNA and an HCV genotype 2 or 3, whatever their liver histology score.

PEG-IFN-α 2a QW + ribavirin QD

Treatment Duration : Genotypes 2/3



(Hadziyannis et al., EASL 2002)

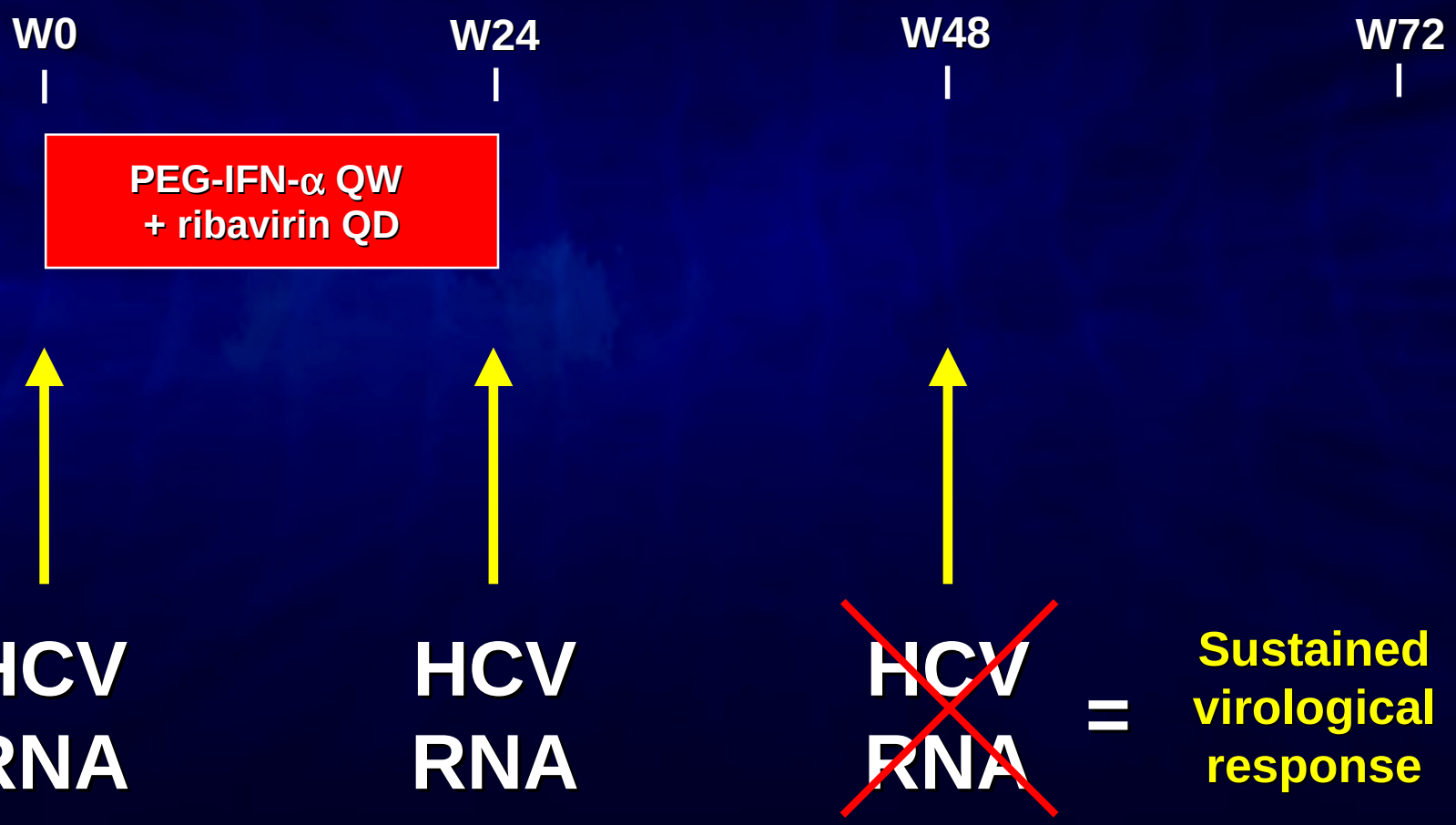
Summary

- **In the patients infected with HCV genotypes 2 or 3, 48 weeks of treatment do not do better than 24 weeks of treatment.**

=> The patients infected with HCV genotypes 2 and 3 must be treated with the combination of PEG-IFN-α plus ribavirin for 24 weeks.

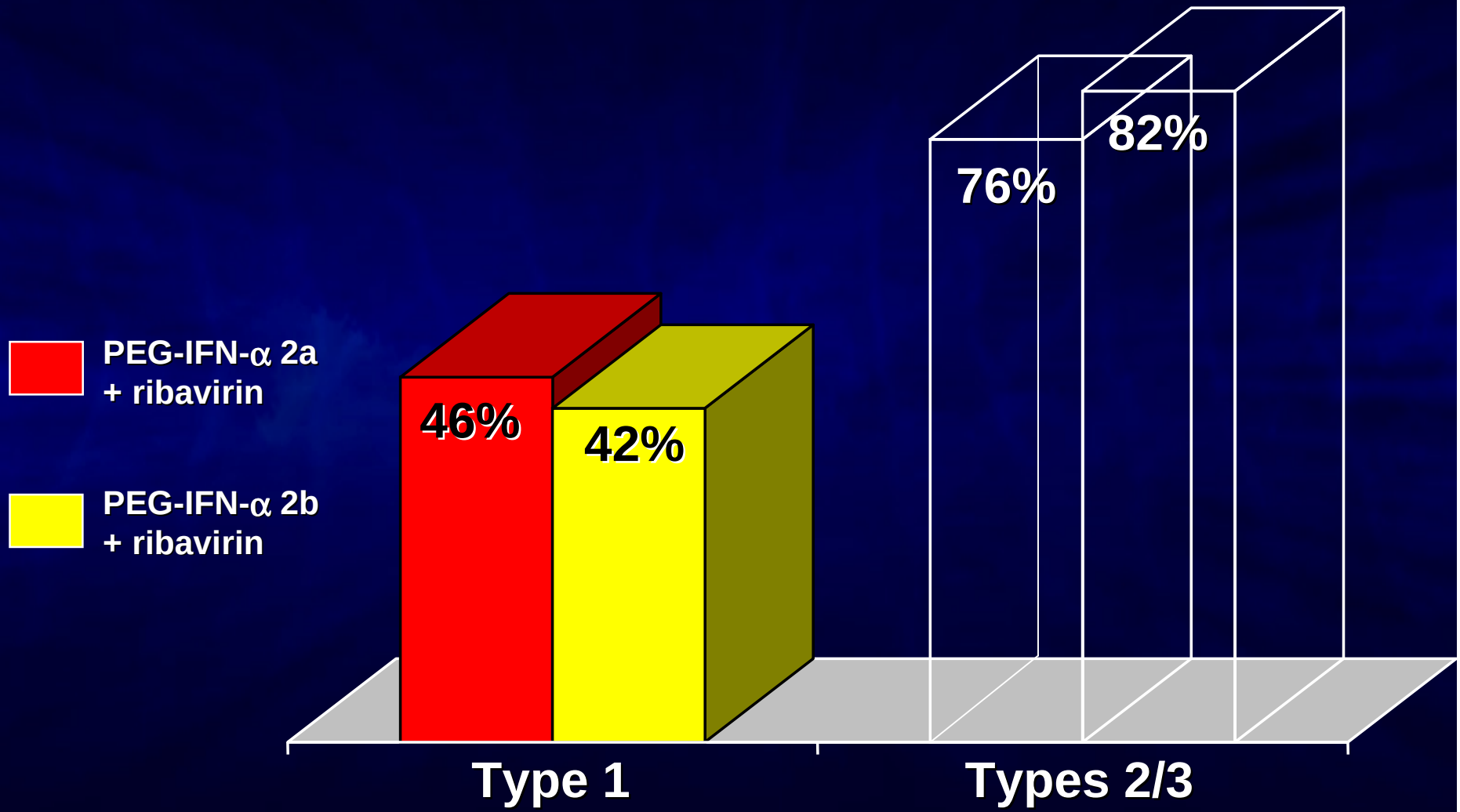
Assessment of Virological Response

Genotypes 2 and 3



PEG-IFN-α QW + ribavirin QD

Naive patients (48 weeks)



(Manns et al., Lancet 2001;358:958-65 ; Fried et al., submitted)

Benefits from Therapy

- **YES…** if moderate to severe necro-inflammatory activity and/or fibrosis.

- **NO…** if “mild“ lesions.

(International Consensus Conference on Hepatitis C, Paris, Février 1999)

Résumé

- The administration of PEG-IFN-α and ribavirin for 48 weeks allows for a sustained virological response in less than half of the patients infected with HCV genotype 1.

- In this context, the potential benefits of therapy must be evaluated according to baseline liver biopsy.

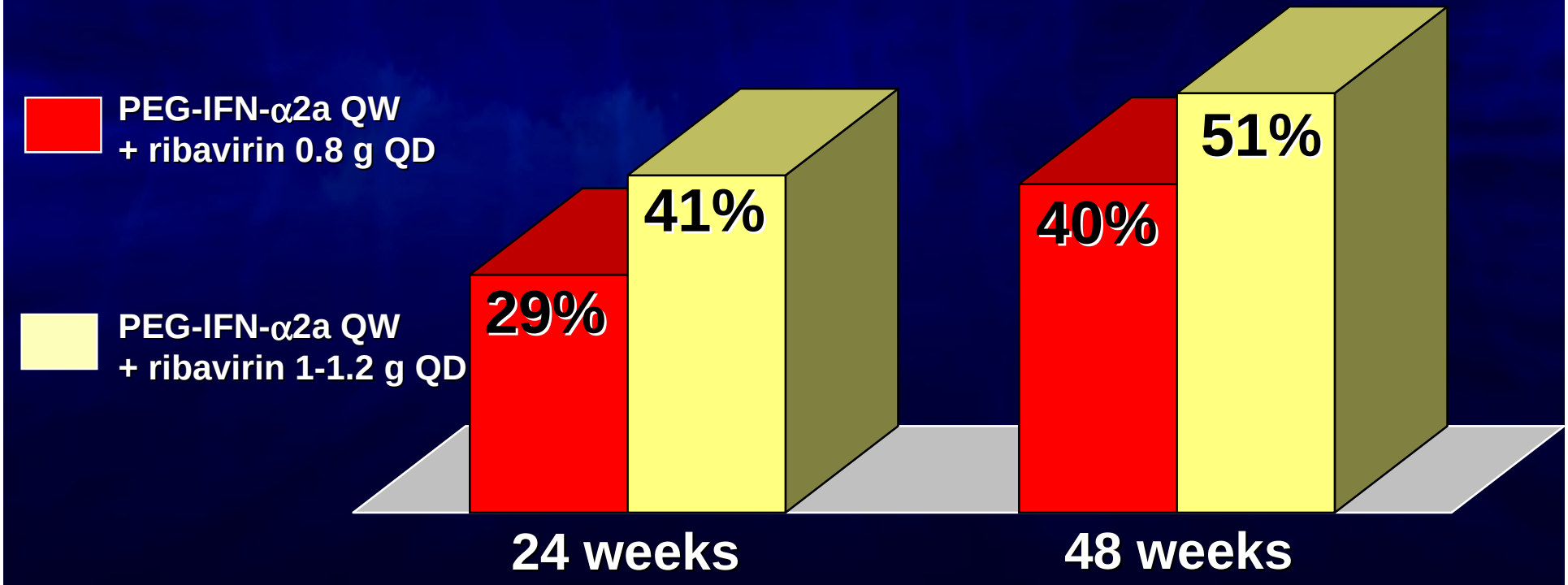
=> in the patients infected with HCV genotype 1 (and, by extension, 4, 5 or 6) :

. Liver biopsy helps in making a decision to treat,

. Antiviral treatment must be proposed if the natural prognosis is pejorative.

PEG-IFN-α 2a QW + ribavirin DQD

Treatment Duration : Genotype 1



(Hadziyannis et al., EASL 2002)

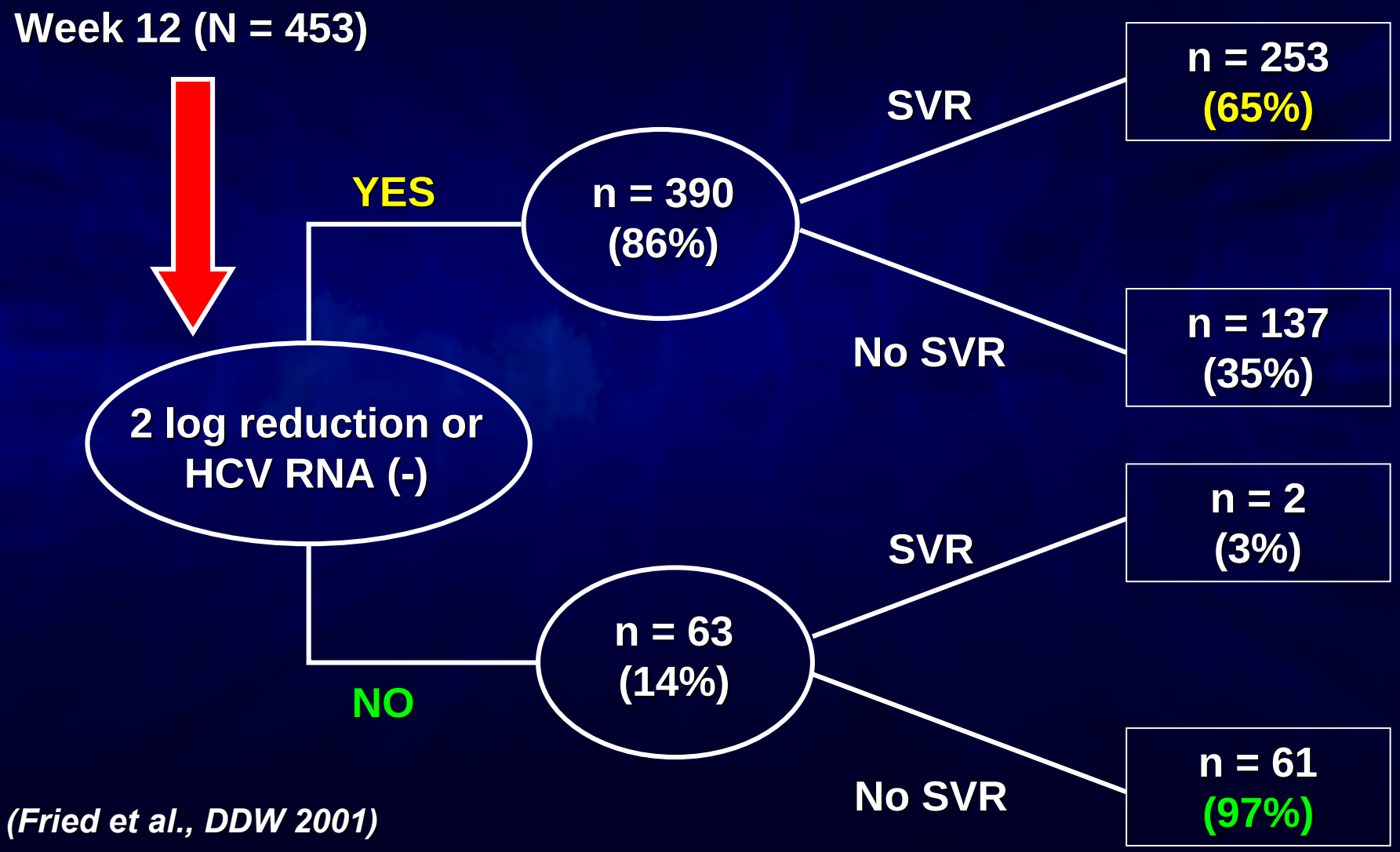
Optimal Treatment Duration

- 48 weeks of treatment do significantly better than 24 weeks.

=> The patients with HCV genotype 1 (and 4, 5, 6) infection and an indication for treatment should receive the combination of PEG-IFN-α and ribavirin for 48 weeks.

PEG-IFN-α2a QW + ribavirin QD

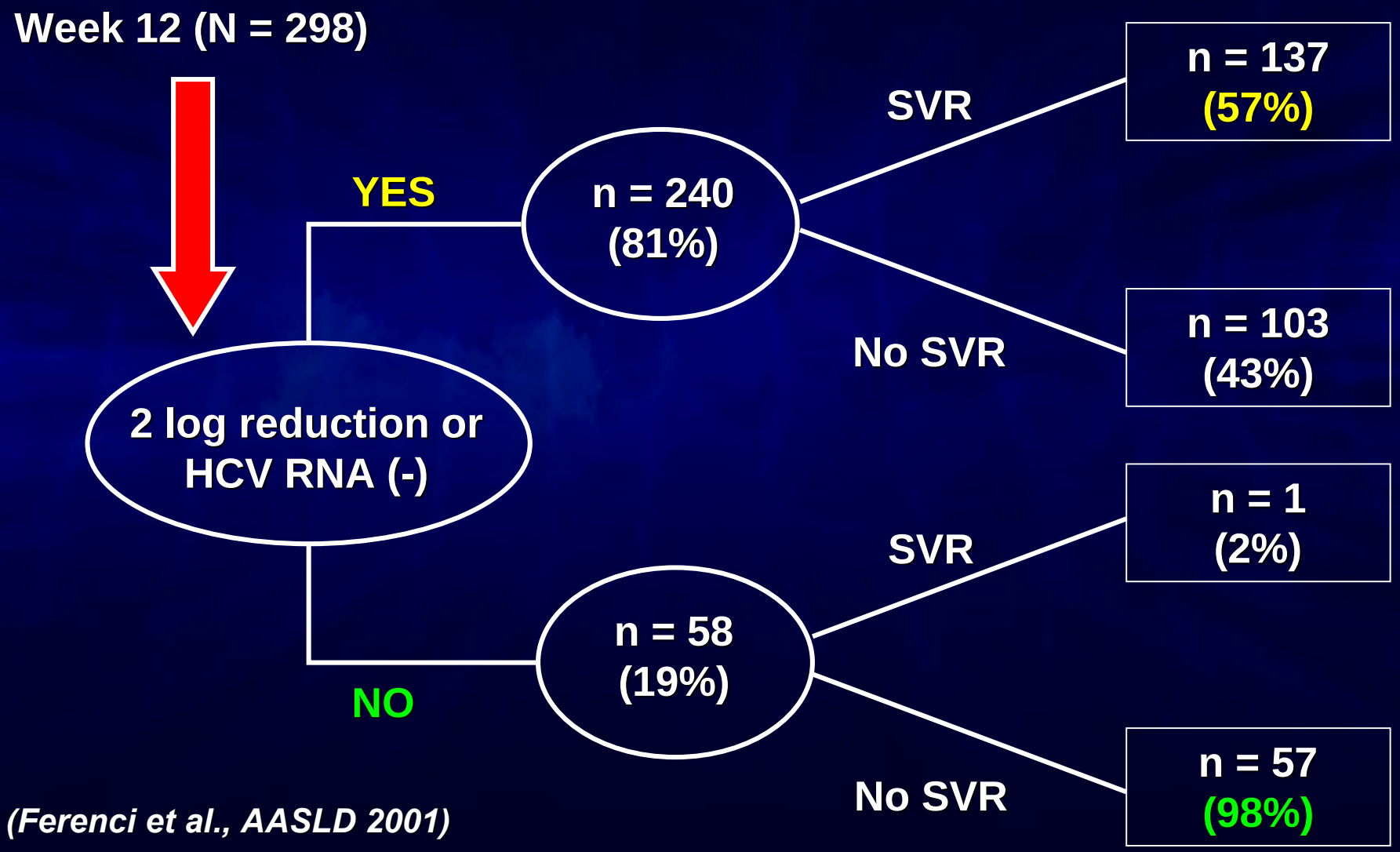
All genotypes



(Fried et al., DDW 2001)

PEG-IFN-α2a QW + ribavirin QD

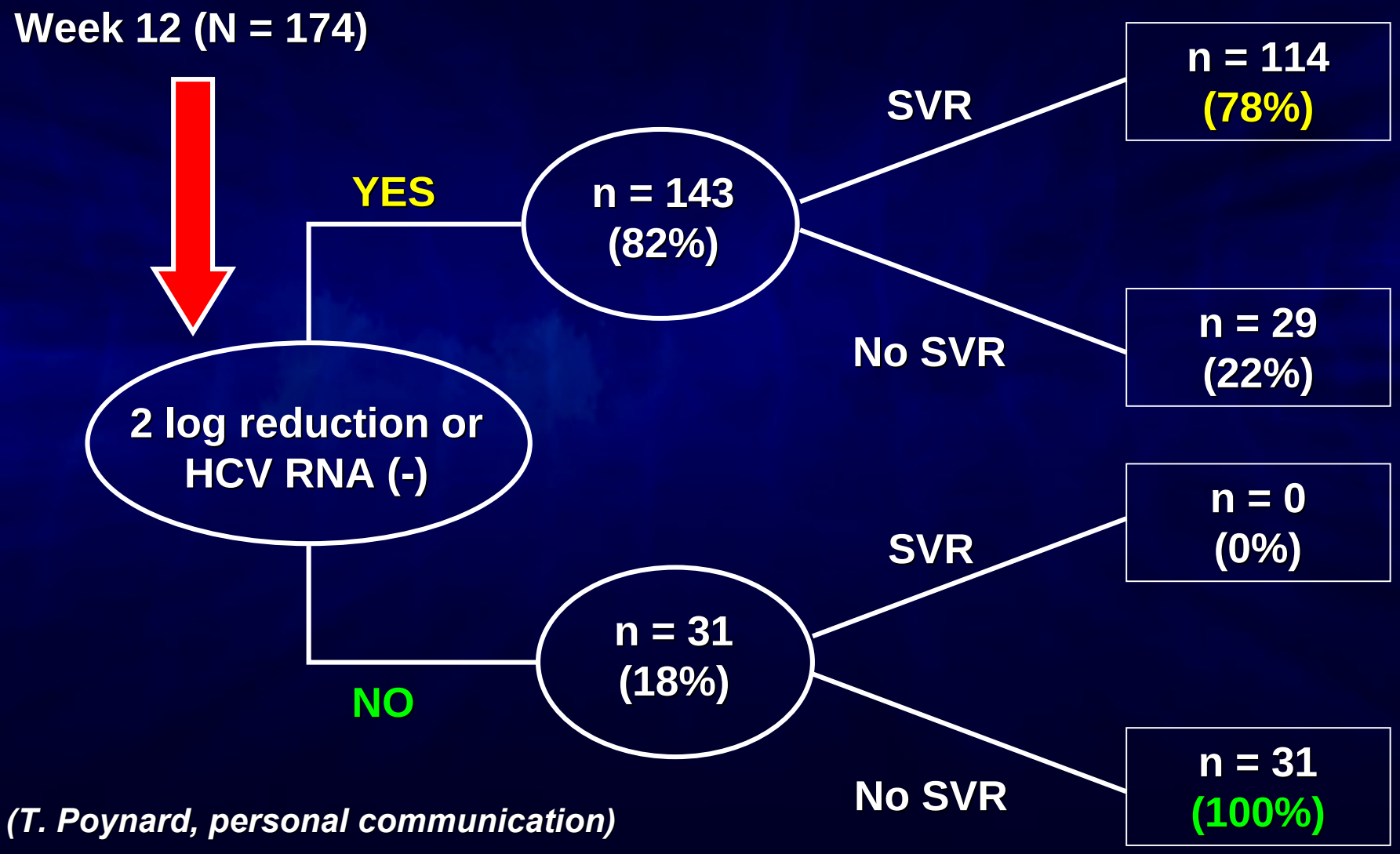
Genotype 1



(Ferenci et al., AASLD 2001)

PEG-IFN-α2b QW + ribavirin QD

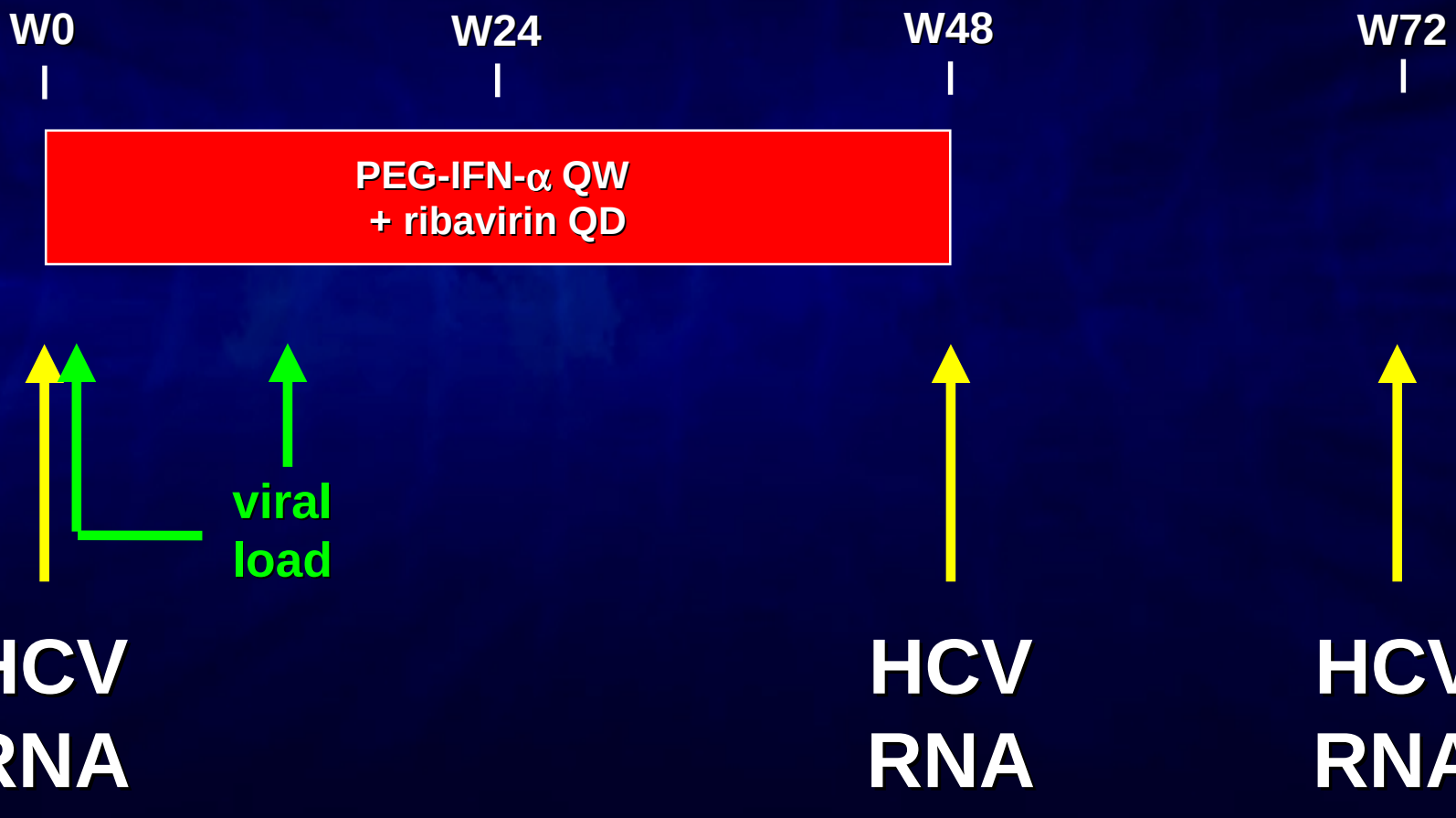
All genotypes



(T. Poynard, personal communication)

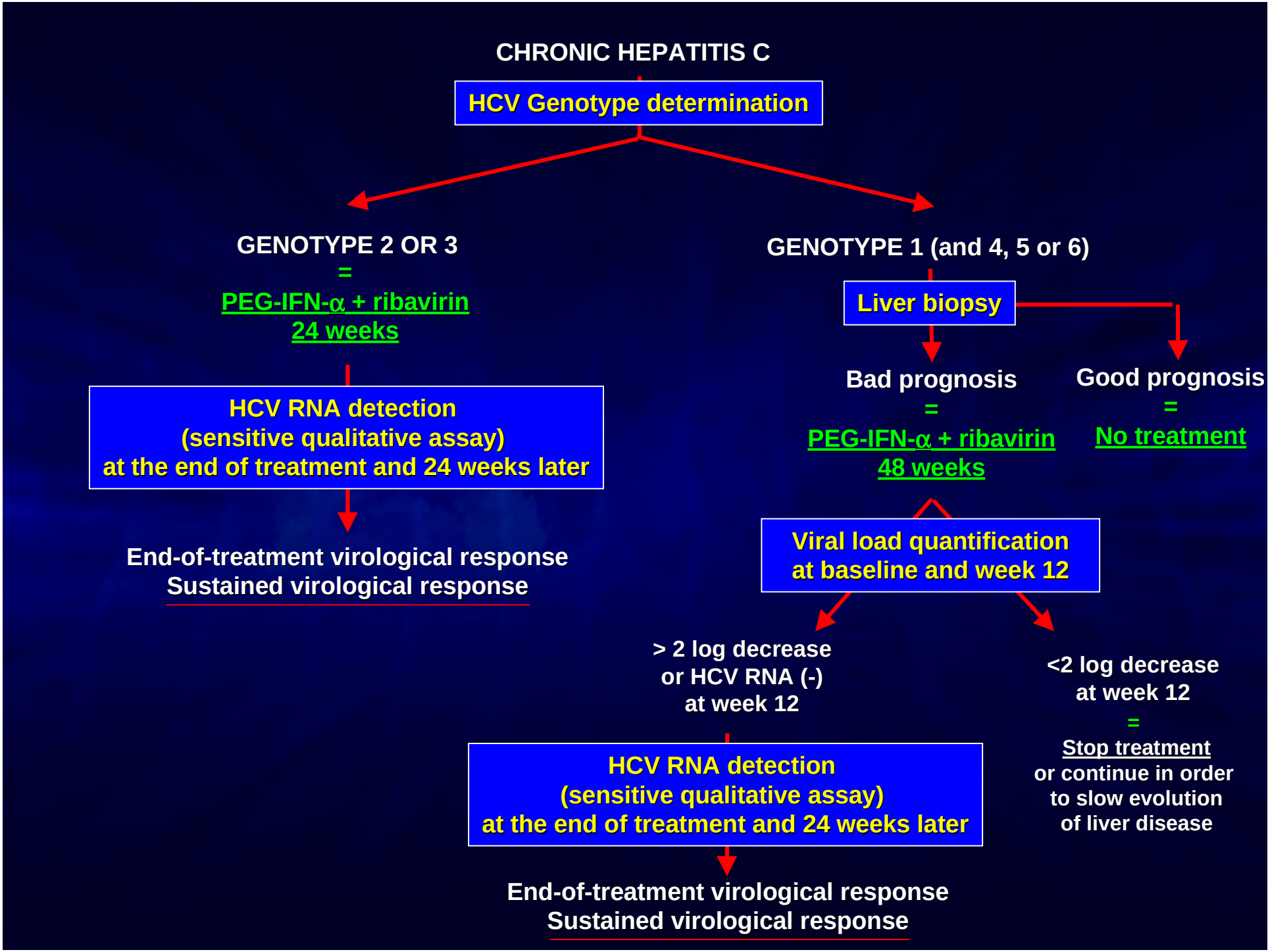
Assessment of Virological Response

Genotypes 1 (and 4, 5, 6)



IV

Treatment Algorithm


CHRONIC HEPATITIS C
HCV Genotype determination
GENOTYPE 2 OR 3
=
PEG-IFN-α + ribavirin
24 weeks
HCV RNA detection
(sensitive qualitative assay)
at the end of treatment and 24 weeks later
End-of-treatment virological response
Sustained virological response
GENOTYPE 1 (and 4, 5 or 6)
Liver biopsy
Bad prognosis
=
PEG-IFN-α + ribavirin
48 weeks
Good prognosis
=
No treatment
Viral load quantification
at baseline and week 12
> 2 log decrease
or HCV RNA (-)
at week 12
<2 log decrease
at week 12
=
Stop treatment
or continue in order
to slow evolution
of liver disease
HCV RNA detection
(sensitive qualitative assay)
at the end of treatment and 24 weeks later
End-of-treatment virological response
Sustained virological response