HCV diagnosis and monitoring in the era of direct-acting antiviral agents

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Material to be covered

- Diagnosis
  - Including sampling
- Pre-Treatment Assessment
- On-treatment Monitoring
  - Stopping rules
  - Response Guided Therapy

Changing therapies

- Evolution of different regimens ….
  - PEG RV
  - PEG RV PI 1st gen
  - PEG RV DAAs
  - DAAs +/- RV
- Has led to evolution of laboratory requirements

Diagnosis

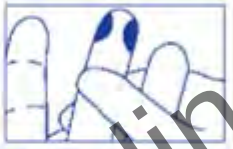
- Venous blood sample
- Dried blood spot

Blood Spot Sample Collection

1) Disposable gloves should be worn for the whole procedure to protect you and to protect the sample from degradation.

2) Ensure the patients hands are clean. If the hands are cold ask the patient to rub them together. It is important the puncture site is warm.

3) Identify and clean the puncture site:
- Use one of the outer 3 fingers
- Avoid finger pad and nail bed
- Clean site with alcohol wipe then dry



4) Break the Seal by Twisting.

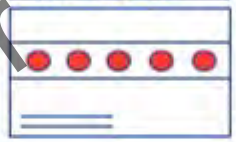
5) Hold the lancet with two fingers and place at the puncture site.

6) Gently apply pressure until the lancet is activated. A click sound will be heard when this has happened.

7) Safely discard the lancet in a sharps container.

8) Spot one blood drop in each of the 5 circles on the card. To ensure enough blood flow:
- Hold the puncture site down
- Apply intermittent pressure

Avoid strong repetitive pressure (milking), it can damage the area.



9) Leave the card to dry for a minimum of **2 hours**.

10) Place the card along with **one bag of dessicant** in the plastic bag attached to the request form.

11) Check the request form and the dried blood spot card have had all the required details entered and place the card in the envelope provided and return to the laboratory.

REMOVE COVERING STRIP

PLACE SPECIMEN IN BAG

FOLD TOP OVER TO SEAL

What happens in the lab

- Dried blood spot – containing paper immersed in buffer
- Elution overnight
- Eluate handled as per VBS (diluted)
- Anti-HCV testing
- HCV RNA testing

DBS Pros and Cons

- Allows sampling in settings where VBS may not be possible
- Patient preference
- Easy transport of samples to lab
- Can test for other analytes eg HBsAg, anti-HIV
- Extra cost in processing
- EIA needs to be recalibrated
- Less sensitive – HCV RNA > 1000 iu/ml

Anti-HCV POSITIVE

- Evidence of infection at some time
- Gives no indication as to when infection occurred
- Gives no indication as to whether infection was cleared or is still present

Virus Detection

- Genome – e.g. Reverse Transcriptase PCR
  - More expensive, more sensitive
- Core Antigen
  - Less expensive, less sensitive

Diagnosis

- Anti-HCV positive
- HCV RNA (or antigen) positive
- = current infection therefore eligible for therapy

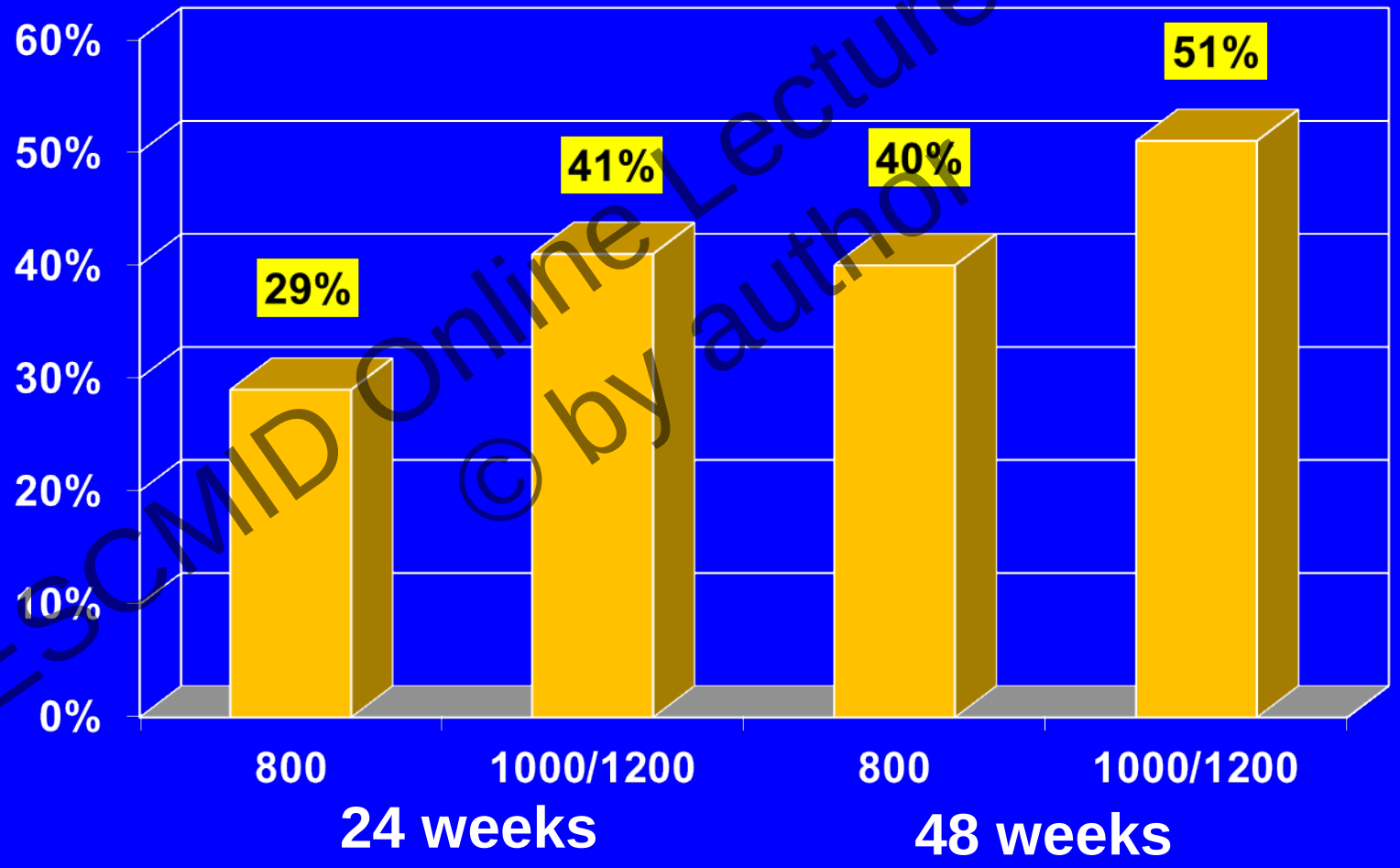
Anti-HCV: Negative

- No evidence of infection with HCV
- BUT - be aware of possible false negatives
  - if infection very recent (window period)
  - if patient immunosuppressed at time of infection
- ? Role for HCV RNA as primary test
- ? Other modes of diagnosis of acute infection

Pre-Treatment assessment

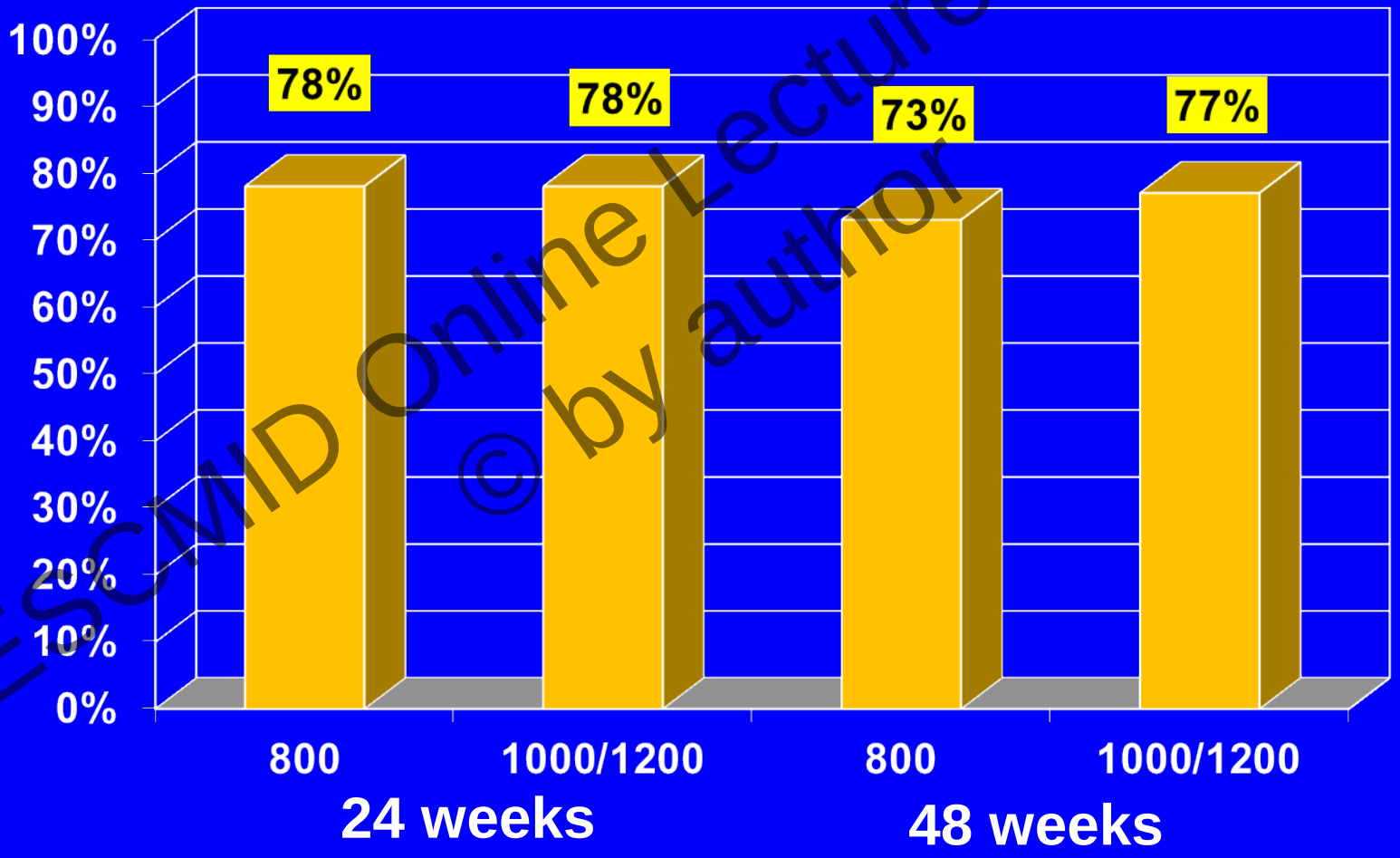
- Genotype

PEG-IFN alpha2a & Ribavirin Genotype 1



Hadziyannis EASL 2002

PEG-IFN alpha2a & Ribavirin
Genotype non-1 (mainly 2 & 3)



Hadziyannis EASL 2002

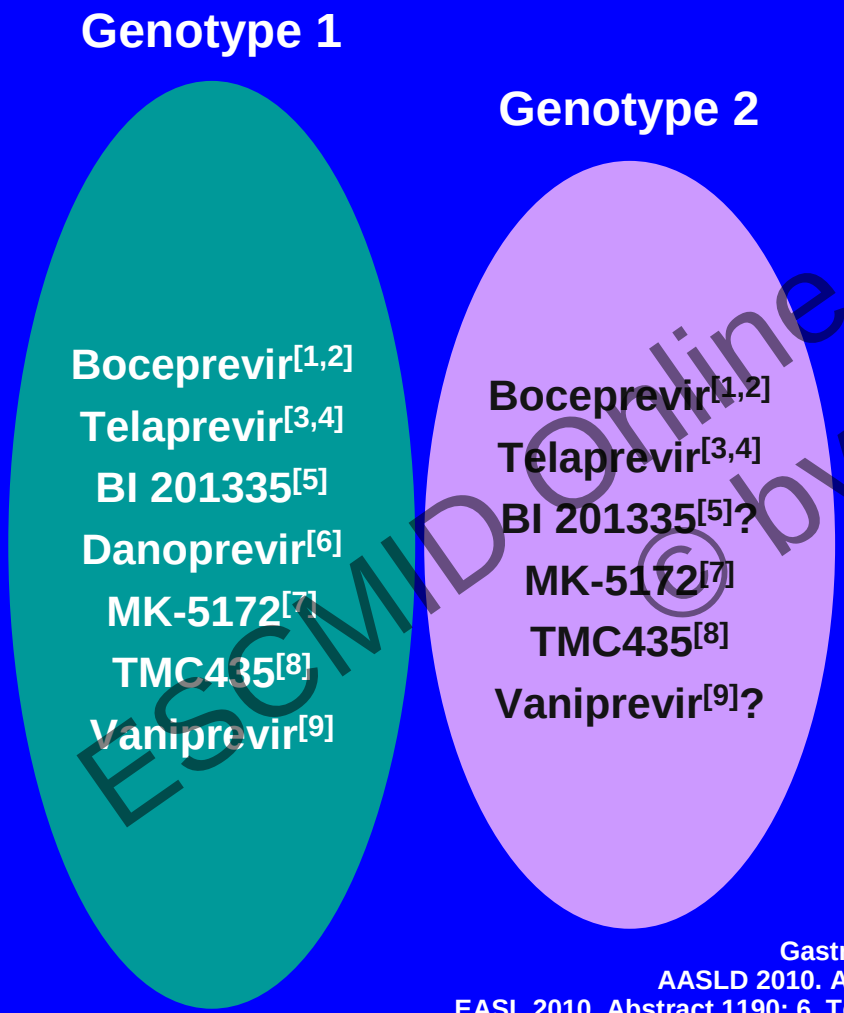
Activity of protease inhibitors by HCV genotype

Genotype 1

Boceprevir[1,2]
Telaprevir[3,4]
BI 201335[5]
Danoprevir[6]
MK-5172[7]
TMC435[8]
Vaniprevir[9]

1. Poordad F, et al. AASLD 2010. Abstract LB-4; 2. Pawlotsky JM, et al. Gastroenterology 2011 [epub ahead of print]. Abstract 820; 3. Jacobson IM, et al. AASLD 2010. Abstract 211; 4. Foster G, et al. EASL 2010. Abstract 57; 5. Sulkowski M, et al. EASL 2010. Abstract 1190; 6. Terrault N, et al. AASLD 2010. Abstract 32; 7. Brainard DM, et al. AASLD 2010. Abstract 807; 8. Fried M, et al. AASLD 2010. Abstract LB-5; 9. Manns MP, et al. AASLD 2010. Abstract 82.
Zeuzem S. CCO slide set

Activity of protease inhibitors by HCV genotype



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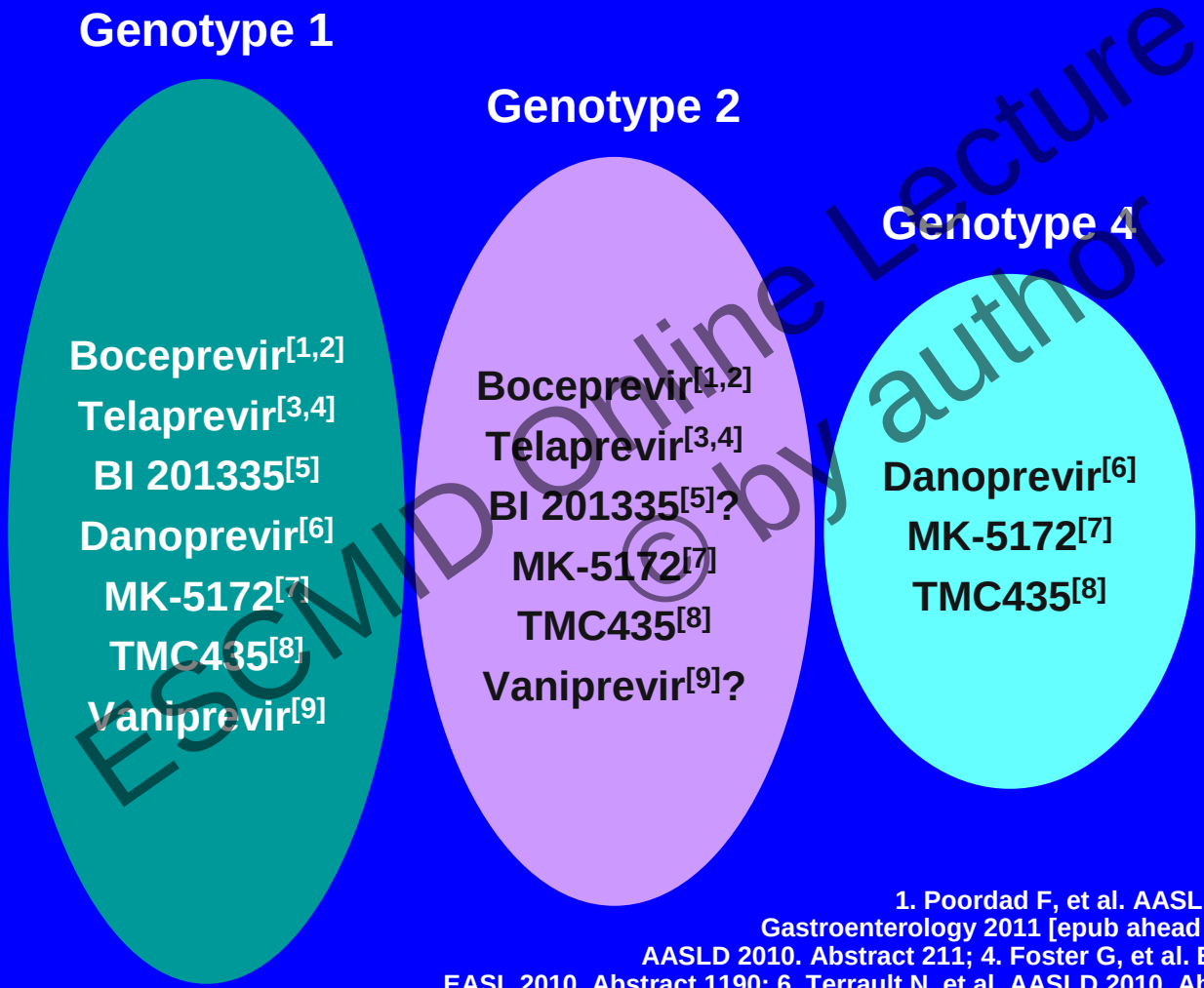

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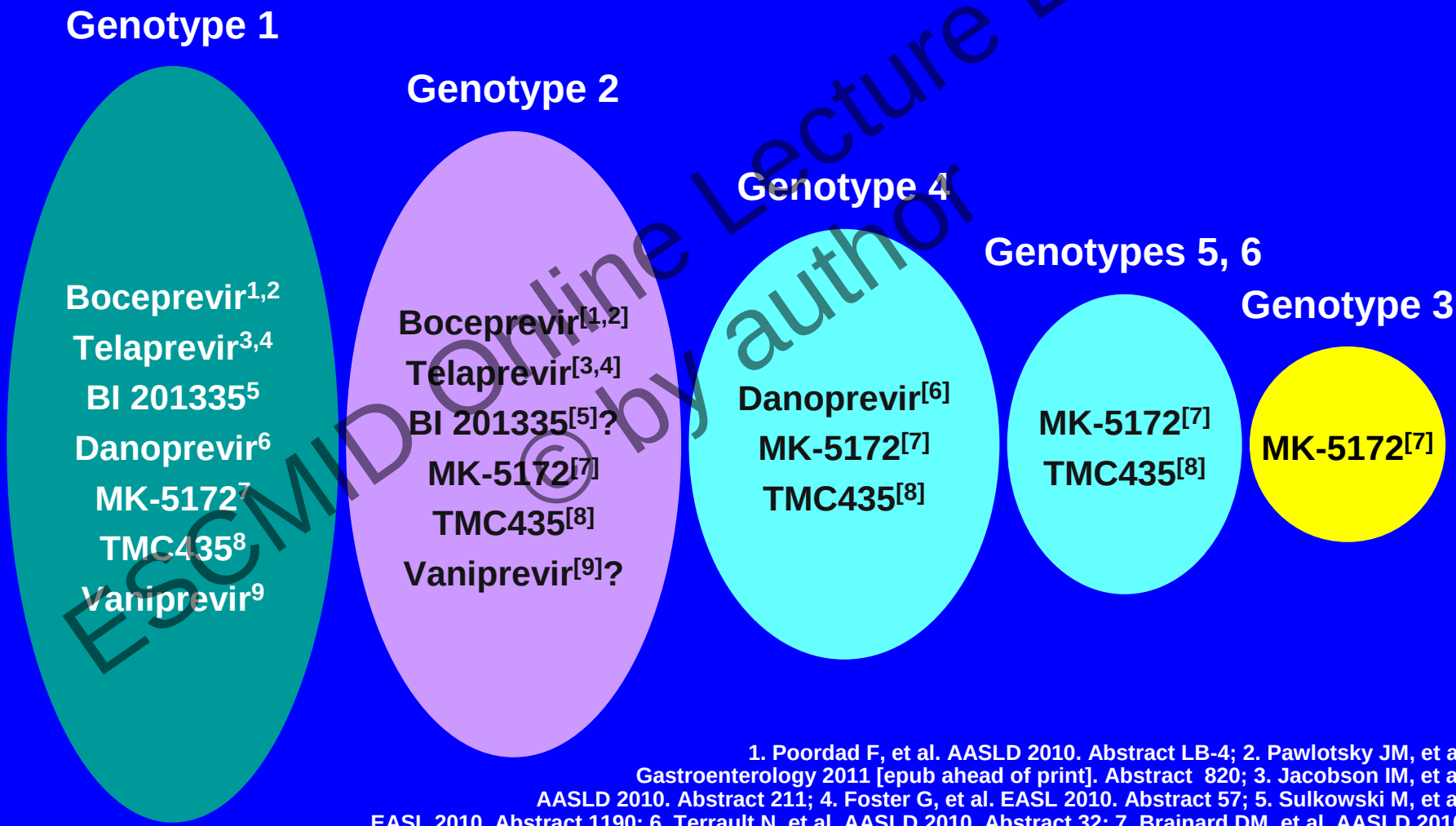
Activity of protease inhibitors by HCV genotype



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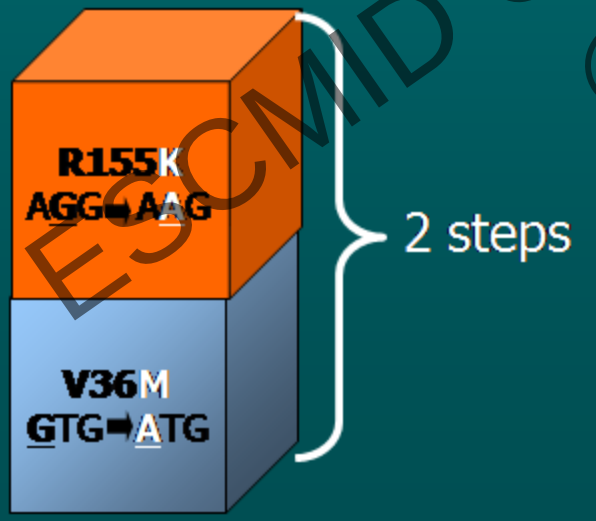
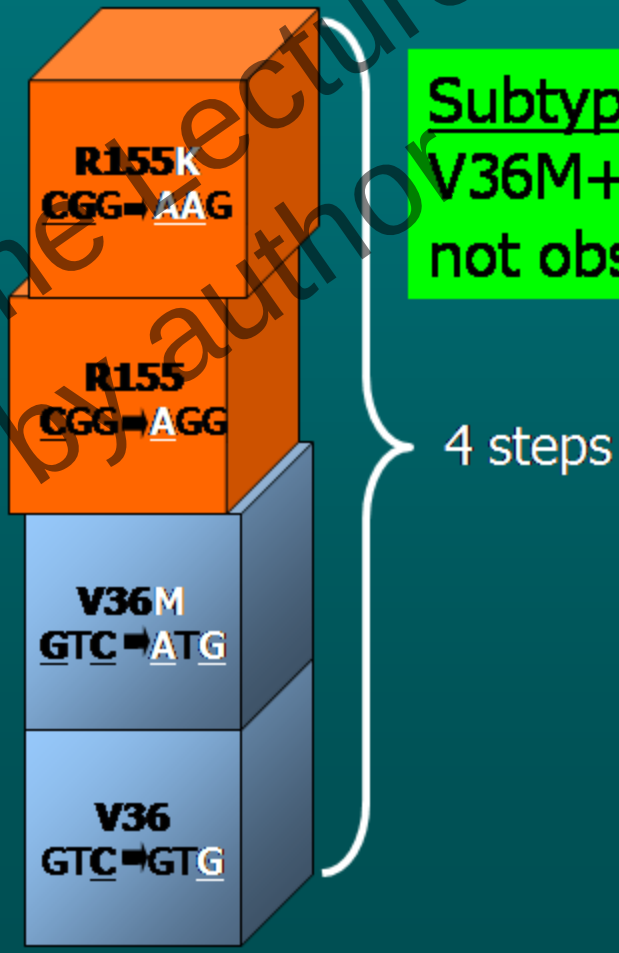
Pre-Treatment assessment

- Genotype
  - PEG + RV
  - PEG + RV + PIs
- Sub-genotyping

Clinical Implications of Genetic Barrier to Resistance – Acquisition of NS3 Inhibitor Resistant Variant V36M+R155K

Subtype 1a
V36M+R155K variant observed clinically[1,2]





Subtype 1b
V36M+R155K variant not observed clinically

McHutchison JG. *N Engl J Med.* 2009; 360; 18 1827-1838
Hezode C. *N Engl J Med.* 2009; 360; 18 1839-1850

PIs: Resistance

- May be inherent i.e. not all genotypes are sensitive
- Genetic barriers vary according to subtype
  - How good are our typing assays?
  - J Clin Virol 2014; 60: 301-304
    - 1052 samples genotyped
    - 54 samples reported only as “genotype 1”
    - Sequencing resolved into 1a or 1b

Pre-Treatment assessment

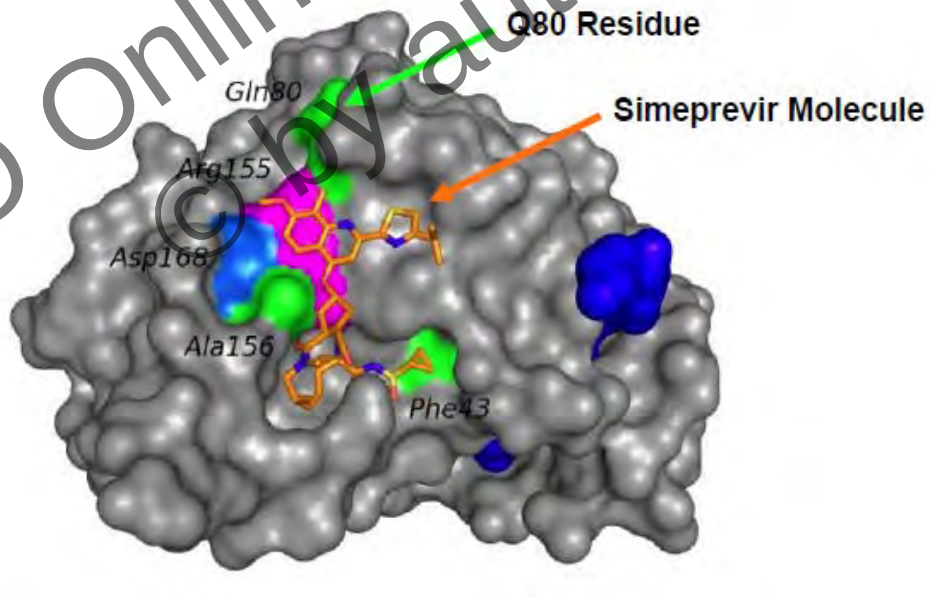
- Genotype
  - PEG + RV
  - PEG + RV + PIs
- Sub-genotyping
  - PEG + RV + PIs
- Strain typing

Q80K – individualising treatment

Naturally occurring polymorphism in the viral amino acid sequence

Q 80 K

Wild-type amino acid *Amino acid position* *Mutated amino acid*



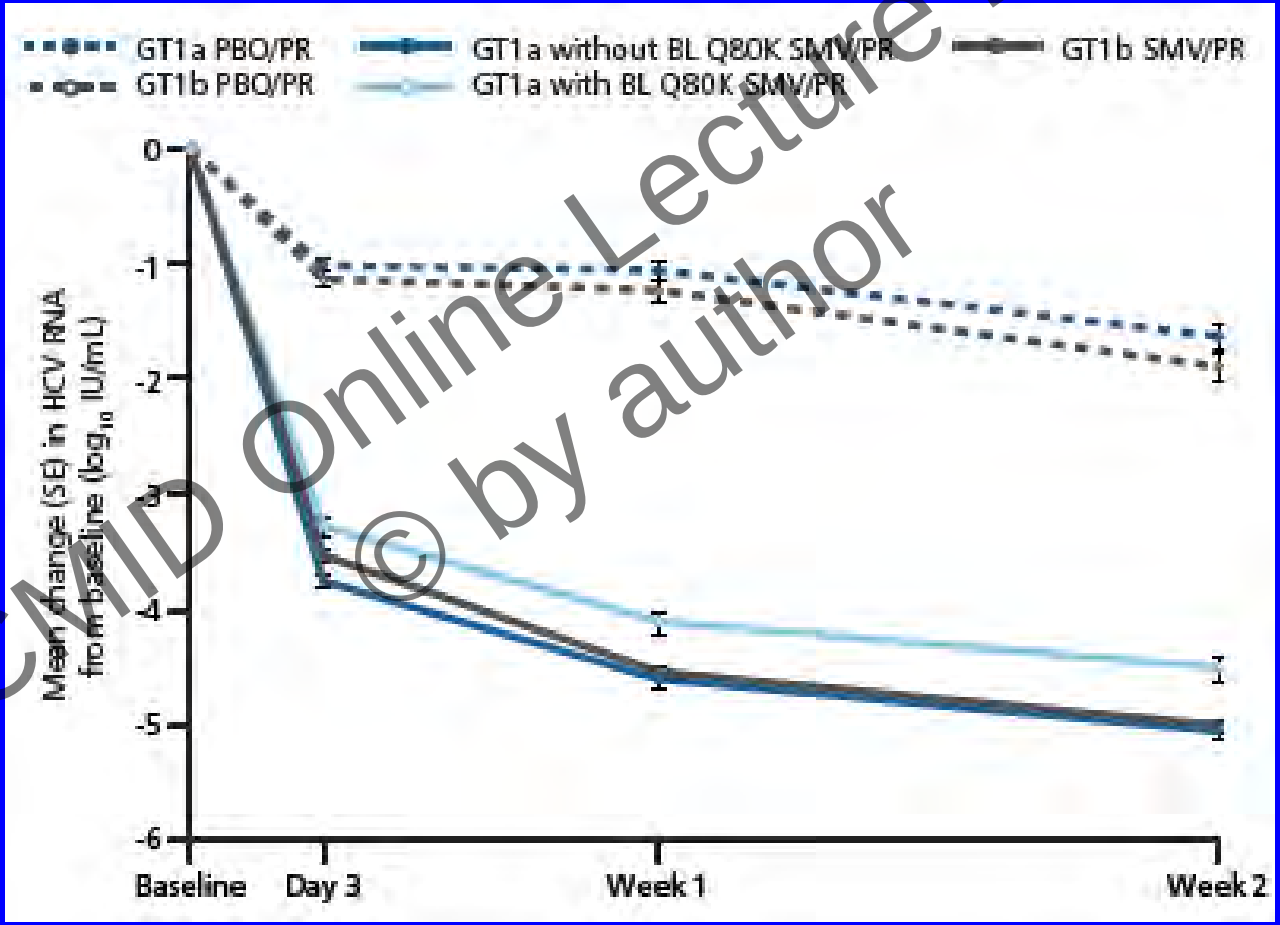
Sherwat, A. FDA Antiviral Drugs Advisory Committee Meeting. 2013: http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/AntiviralDrugsAdvisoryCommittee/UCM375113.pdf

The Q80K polymorphism is less prevalent in Europe compared to the US

n/N (%)	All HCV genotype 1	HCV genotype 1a	HCV genotype 1b
Overall	274/2007 (13.7)	269/911 (29.5)	5/1096 (0.5)
Europe	76/1254 (6.1)	73/377 (19.4)	3/877 (0.3)
North America	185/538 (34.4)	185/385 (48.1)	0/153 (0)
South America	2/60 (3.3)	2/22 (9.1)	0/38 (0)

1. Jacobson I, et al. AASLD 2013. Poster 1122
2. Lenz O, et al. AASLD 2013. Poster 1101

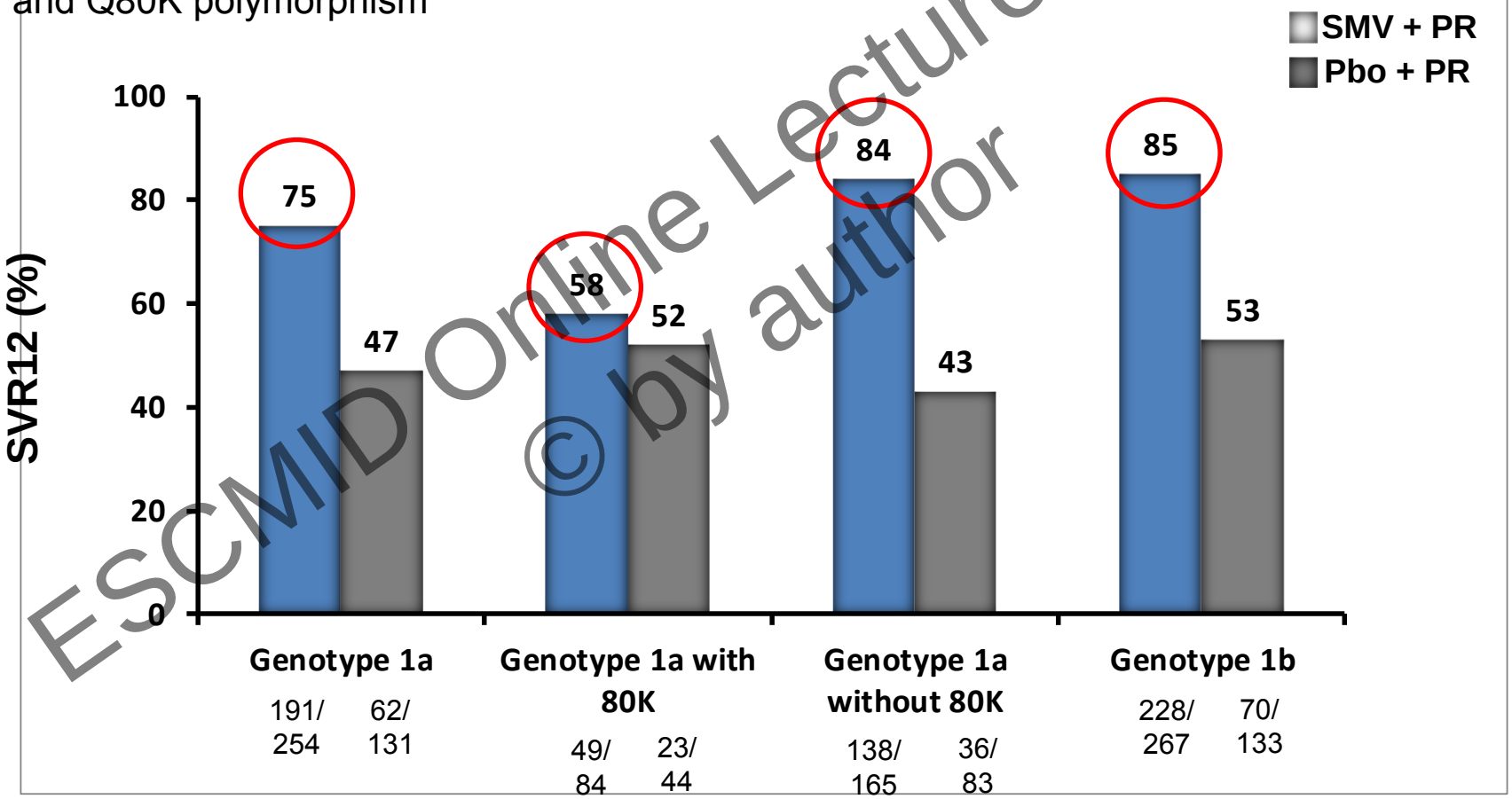
Early Viral Decay and Q80K



Lenz O. et al. Presented at EASL 2014. Poster 221

Q80K is a predictor of response: Treatment Naive

SMV + PR: pooled QUEST 1 and 2 – SVR12 by HCV genotype and Q80K polymorphism



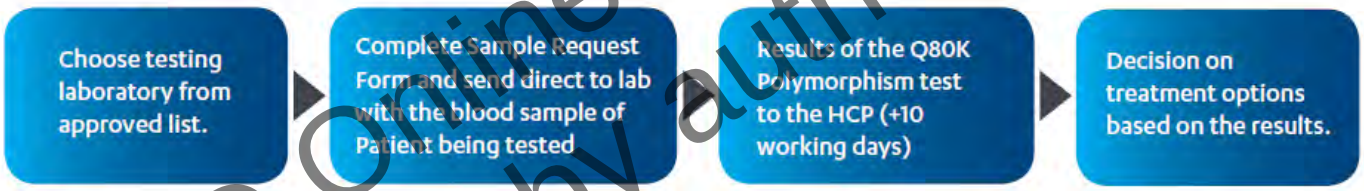
How to test for Q80K?

Process for Q80K Polymorphism Test

The Q80K polymorphism test is strongly recommended as it is likely to be a predictor of response for treatment options for patients starting treatment. The test should be considered as a routine part of the workup for patients with Hepatitis C Genotype 1.[1]

Janssen understands that the cost and logistics of providing this test may be challenging for some hospitals and are committed to minimising the potential impact of providing this test.

If you decide to test for Q80K Polymorphism in G1 patients, please complete the Janssen T&C form, provided by the Janssen Account Manager. You can use the following process to access the test:



- The provision of the Q80K test by Janssen does not require you to use any of the company's products or services.
- Janssen will cover the costs of the Q80K test for use in NHS treated patients only.
- The tests being requested are for patients with Hepatitis C Genotype 1 and samples are to be sent to one of the laboratories from the approved list.
- Janssen will cover the costs of the testing for patients with Genotype 1 Hepatitis C but not the cost of sample transportation. The responsibility for transporting the sample to the laboratory remains with the hospital. Details of how to send the sample are on the Sample Request Form.

Further information and sample request forms can be obtained via email on **janssenukhepc@its.jnj.com** or by contacting your local Janssen Account Manager.

janssen
PHARMACEUTICAL COMPANIES OF Johnson & Johnson

Pre-Treatment assessment

- Genotype
  - PEG + RV
  - PEG + RV + PIs
- Sub-genotyping
  - PEG + RV + PIs
- Strain typing
  - PEG + RV + Simeprevir

Pre-Treatment assessment

- Genotype/Subtype/Q80K
- Viral load assessment
  - Pre-Rx viral load correlates with likelihood of achieving SVR

On treatment monitoring

- Stopping rules
  - Identify patients in whom continuing therapy is very unlikely to result in SVR ∴ STOP

Stopping rules PEG/RV: Davis et al (Hepatology 2003:38:645)

- Used patients from Manns et al Lancet 2001
- Tested a variety of definitions of EVR
  - 4/12 weeks
  - 1/2/3 log decrease, PCR neg
- Which definition captured highest no. SVRs (sensitivity), whilst excluding highest no. NRs (NPV)?

SVRs (n=273)		NRs (n=238)	
EVR	No EVR	EVR	No EVR
1-log drop at 12 weeks			
273 (100%)	0	144	94 (38%)
2-log drop at 12 weeks			
273 (100%)	0	107	131 (55%)
1-log drop at 4 weeks			
267 (97.8%)	6	113	125 (52.5%)
2-log drop at 4 weeks			
250 (92%)	23	70	168 (71%)

SVRs (n=273)		NRs (n=238)	
EVR	No EVR	EVR	No EVR
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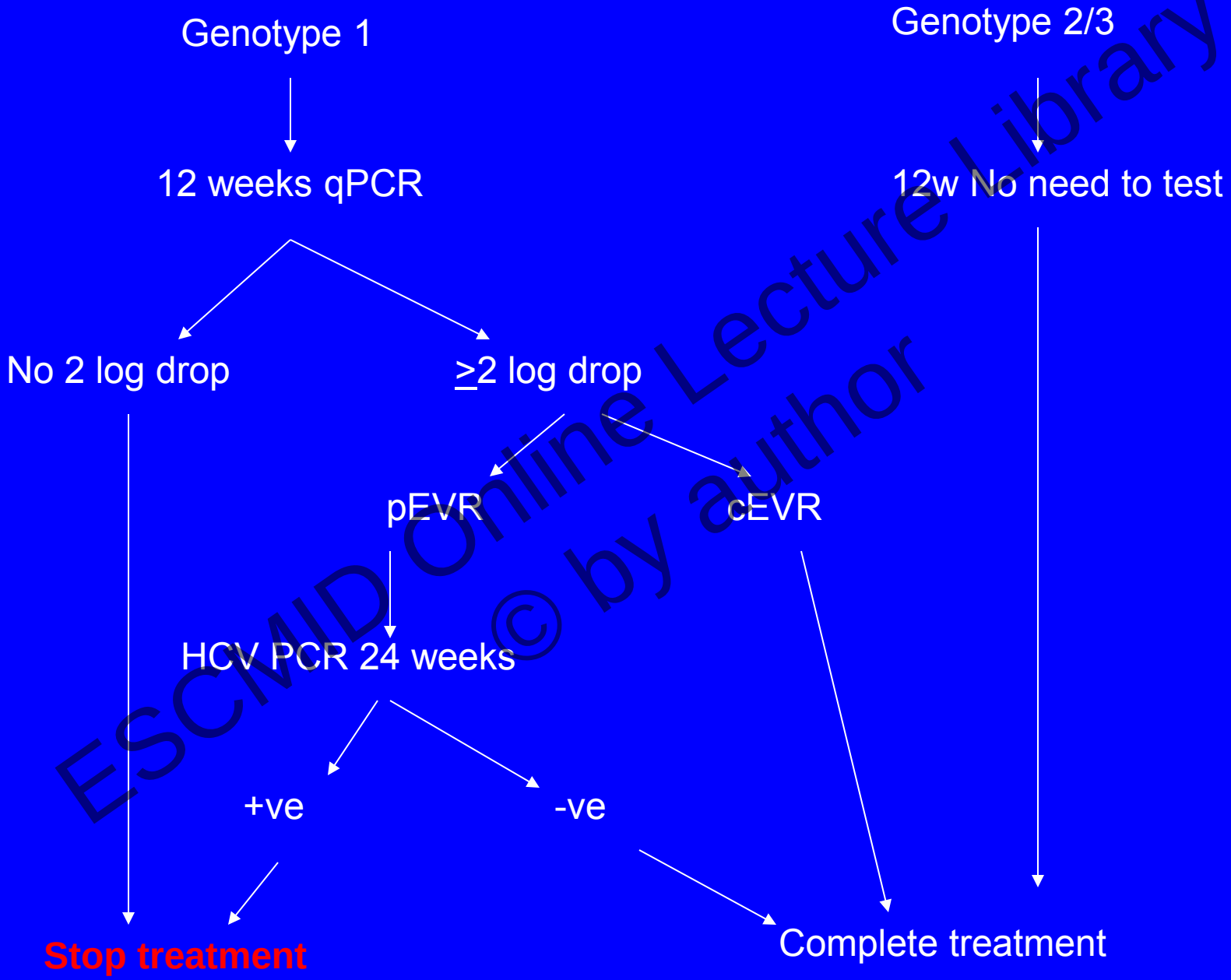
267 (97.8%)	6	113	125 (52.5%)
2-log drop at 4 weeks			
250 (92%)	23	70	168 (71%)

On Rx monitoring: Definitions

- Early Virological Response (EVR)
  - Measured at 12 weeks on therapy
  - Complete (cEVR) = Negative
  - Partial (pEVR) = > 2 log drop
  - Non-response = < 2 log drop



On treatment monitoring

- Stopping rules
- Response Guided Therapy (RGT) rules
  - Stratify treatment regimen according to likelihood of achieving SVR i.e. *shorten* therapy
  - Based on 2 parameters
  - Kinetics of viral loss
  - Baseline viral load

On Rx monitoring: Definitions

- Early Virological Response (EVR)
- Rapid Virological Response (RVR)
  - Assessed at 4 weeks on Rx
  - HCV RNA negative = RVR

What is HCV RNA negativity?

- Different assays have different sensitivities
  - Lower limit of quantification (LLQ)
  - <50? <30? <25? <15? <12? iu/ml
  - Signal may be detectable below LLQ
  - Lower limit of detection (LLD)
  - 9? 12? 15? etc etc
- Original description of RVR was with assays with LLQ around 25-30 iu/ml

Residual virus below LLQ

- Difficult to interpret lab result
  - ? Genuine low level viraemia
  - ? Background noise
- Impacts on likelihood of achieving SVR

Predictors of treatment failure

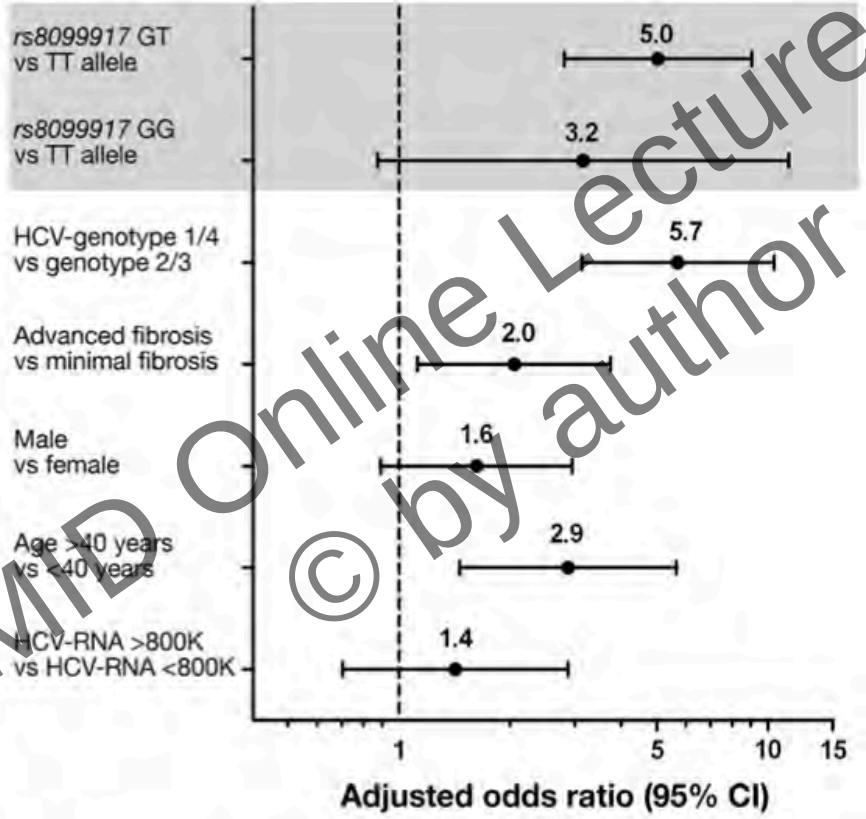


Figure 3. Predictors of failure to respond to pegylated IFN-α and ribavirin therapy. Carriers of the *rs8099917* G-risk genotypes had a higher risk of failing to respond to HCV treatment. ORs were calculated by allele and were adjusted for HCV genotypes, fibrosis stage, sex, age, baseline HCV viral load, and the first 2 ancestry principal components.

RVR vs no RVR

OR is around 7.0

Rauch et.al., Gastroenterology, 2010

RGT: PEG + RV

- Genotype 1 patients who achieve RVR and who had a low pre-Rx viral load – can shorten therapy from 12 to 6 months *without loss of SVR*
- Genotype 2/3 patients who achieve RVR and who had a low pre-Rx viral load – can shorten therapy from 24 to 16 weeks *without loss of SVR*
- ? How low is low?

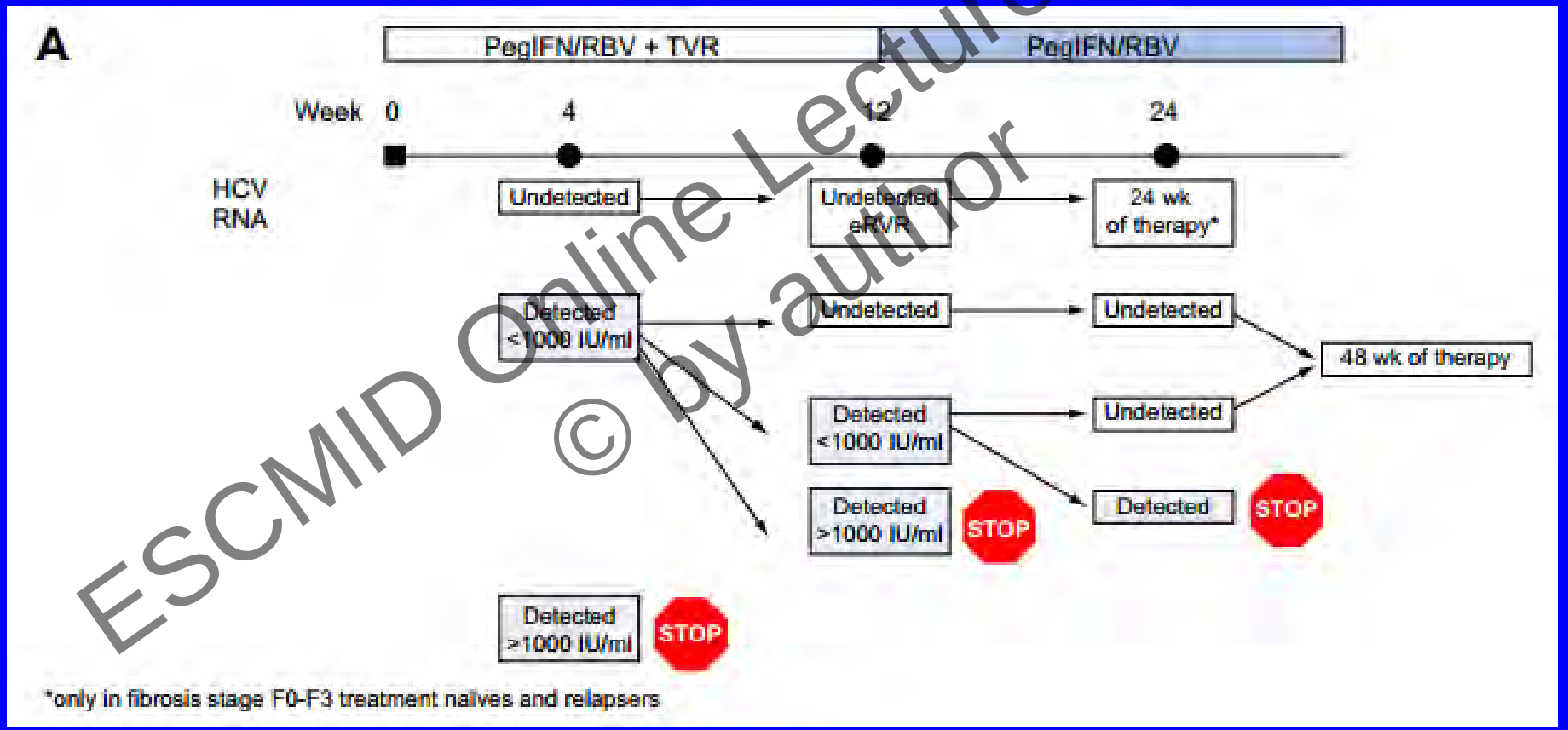
On Rx monitoring: Definitions

- Early Virological Response (EVR)
- Rapid Virological Response (RVR)
- Extended Rapid Virological Response (eRVR)
  - Concept arose with 1st gen PIs
  - To take account of possible breakthrough of PI-resistant virus
  - RNA neg at 4 weeks *AND* 12 weeks of triple therapy = eRVR

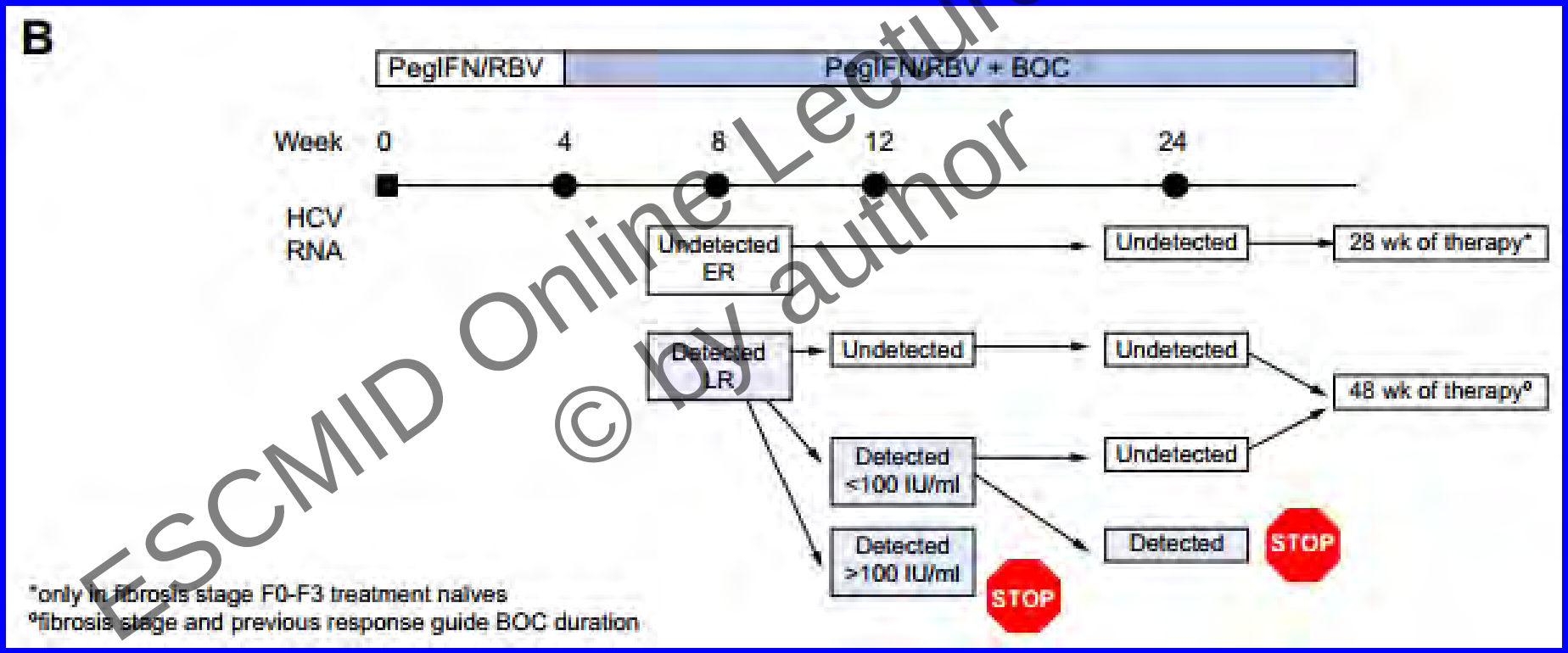
RGT: PEG + RV + TPR/BOC

- Shortened therapy (48 to 24 weeks) in g1 patients treated with triple therapy *if* eRVR was achieved

Teleprevir: Stopping rules and RGT

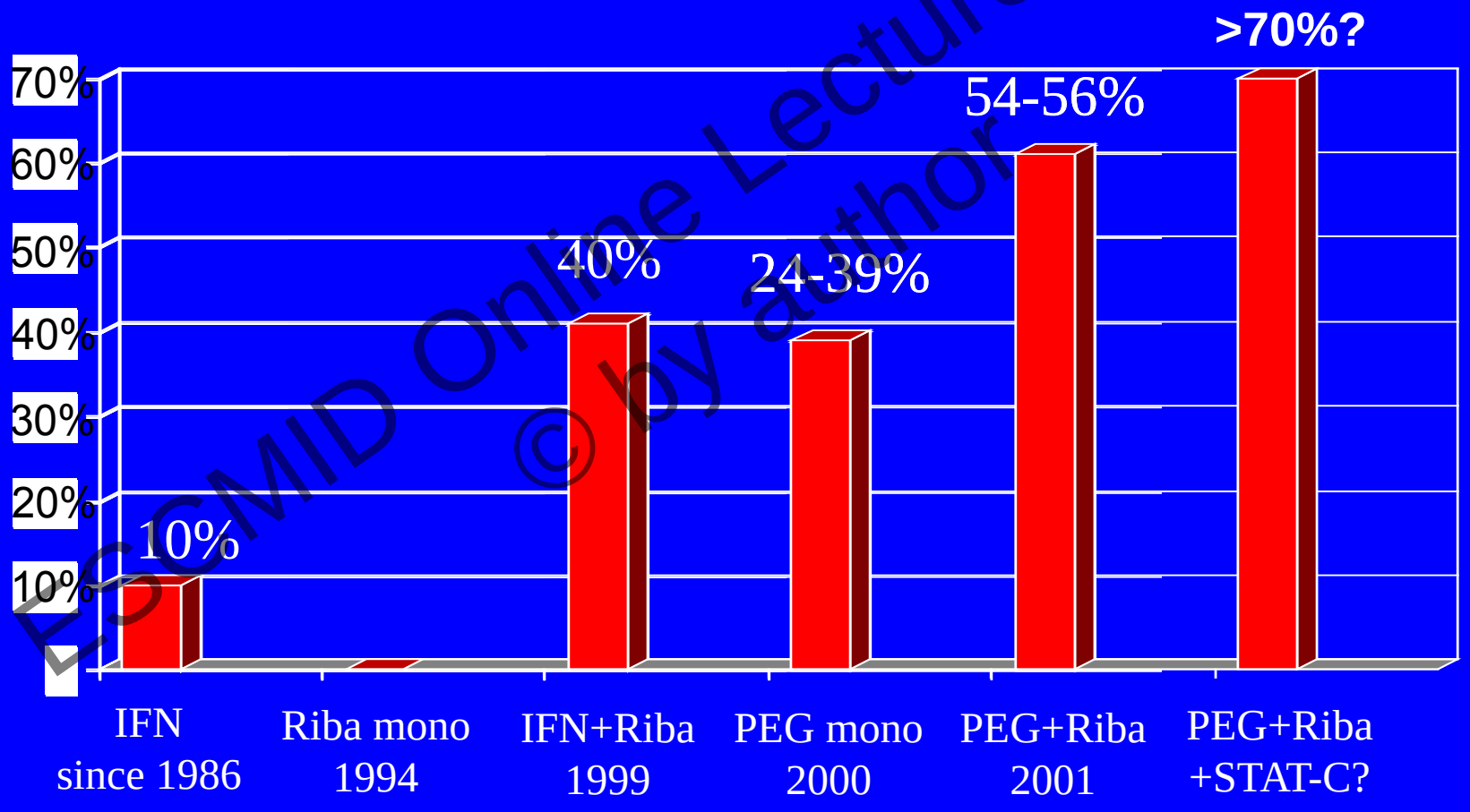


Boceprevir: Stopping rules and RGT



Treatment of Hepatitis C

Sustained Virologic Response (SVR): undetectable HCV RNA at least 6 months after the end of treatment



Hoofnagle et al. NEJM 1986
Brillanti et al. Gastroenterology 1994
McHutchison et al., NEJM 1998
Zeuzem et al., NEJM 2000
Manns et al., Lancet 2001
Fried et al., NEJM 2002

All Oral DAA therapy

The NEW ENGLAND JOURNA

ORIGINAL ARTI

Ledipasvir and Sofosbuv HCV Genotype 1

Nezam Afdhal, M.D., Stefan Zeuzem, M.D., Paul
Norman Gitlin, M.D., Massimo Pueti, M.D., Manuel Romero-Gomez, M.D., Ph.D.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Treatment of HCV with ABT-450/r–Ombitasvir and Dasabuvir with Ribavirin

Jordan J. Feld, M.D., M.P.H., Kris V. Kowdley, M.D., Eoin Coakley, M.D.,

The NEW ENG
JOURNAL of ME

ESTABLISHED IN 1812 APRIL 17, 2014

Ledipasvir and Sofosbuvir for Prev Genotype 1 Infect

Nezam Afdhal, M.D., K. Rajender Reddy, M.D., David R. Nelson, M.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Retreatment of HCV with ABT-450/r–Ombitasvir and Dasabuvir with Ribavirin

Stefan Zeuzem, M.D., Ira M. Jacobson, M.D., Tolga Baykal, M.D.,

The NEW ENGLAND JOURNAL of MEDICIN

ORIGINAL ARTICLE

Ledipasvir and Sofosbuvir for 8 or 1 for Chronic HCV without Cirrh

Kris V. Kowdley, M.D., Stuart C. Gordon, M.D., K. Rajender
Lorenzo Rossaro, M.D., David E. Bernstein, M.D., Eric Lawitz, M.D.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

ABT-450/r–Ombitasvir and Dasabuvir with Ribavirin for Hepatitis C with Cirrhosis

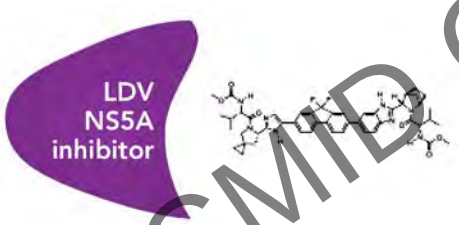
Fred Poordad, M.D., Christophe Hezode, M.D., Roger Trinh, M.D., M.P.H.,
Kris V. Kowdley, M.D., Stefan Zeuzem, M.D., Kosh Agarwal, M.D.

HARVONI▼ is the first and only Single-Tablet Regimen[a] for the majority of HCV GT1 adult patients[1,b]

Excluding those with decompensated cirrhosis, or who are pre- or post-liver transplant[1]

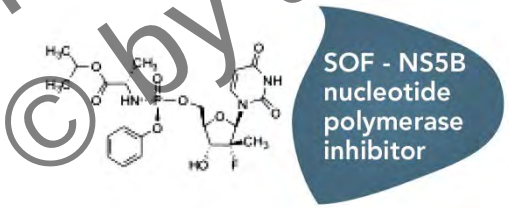
Ledipasvir

- Picomolar potency against HCV GT 1a and 1b[1]
- Effective against NS5B RAV S282T[2]
- Once-daily, oral, 90 mg
- Half-life 47hrs[2]



Sofosbuvir

- Potent antiviral activity against HCV GT 1–6
- Effective against NS5A RAVs[3]
- High barrier to resistance
- Once-daily, oral, 400 mg tablet
- Active metabolite half-life 27hrs[3]



Ledipasvir / sofosbuvir STR

- Once-daily, oral fixed-dose (90/400 mg) combination tablet
- No food effect
- 1,952 patients treated in the phase 3 programme

- Harvoni is indicated for the treatment of chronic hepatitis C (CHC) in adults[1] Genotypes 1, 3 & 4 (GT 3 + RBV)[2]

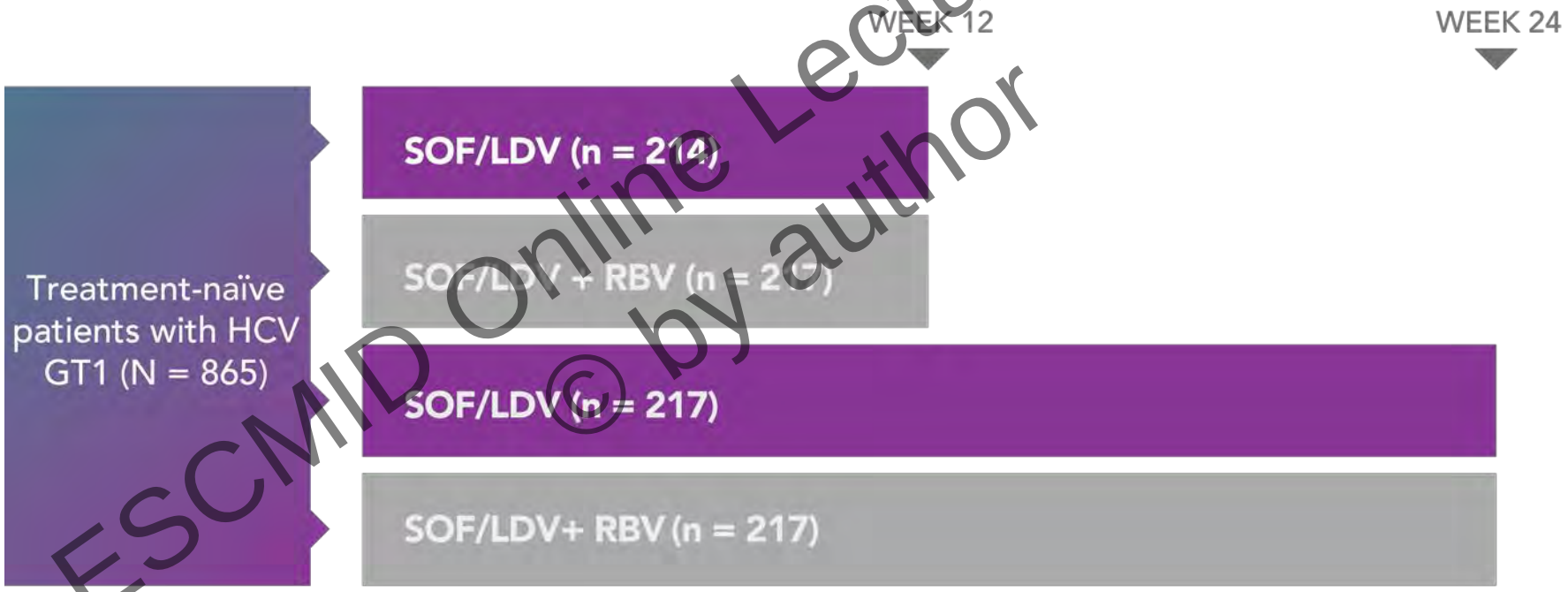
a. Single-tablet regimen is defined throughout this document as one tablet once daily
b. HARVONI offers a single-tablet, RBV-free regimen for the majority of HCV GT1 patients

REFERENCES: 1. Lawitz E, et al. EASL 2011, poster 1219; 2. HARVONI Summary of Product Characteristics, November 2014; 3. SOVALDI Summary of Product Characteristics, June 2014

Study Design – ION-1[1]

Randomised, open-label, Phase III trial in treatment-naïve patients with HCV GT1

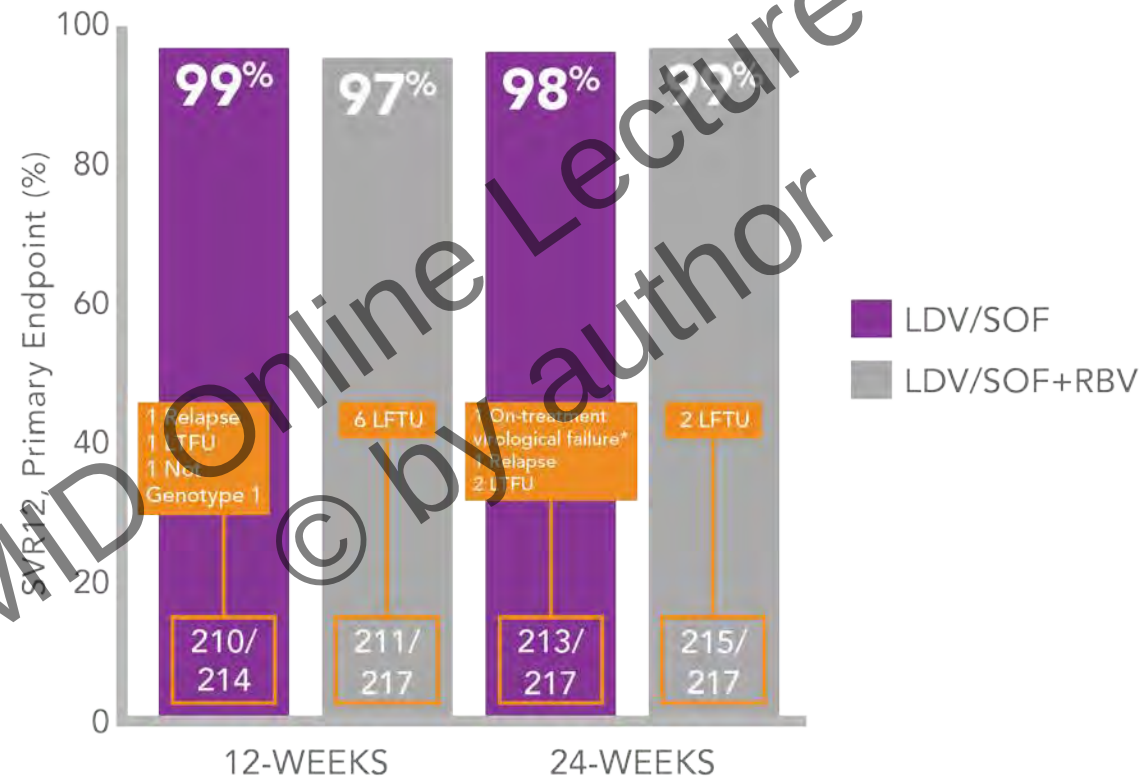
Primary endpoints: SVR12



REFERENCES: 1. HARVONI Summary of Product Characteristics, November 2014

ION-1

Genotype 1 treatment naïve, including cirrhosis, 12 and 24 weeks[1]

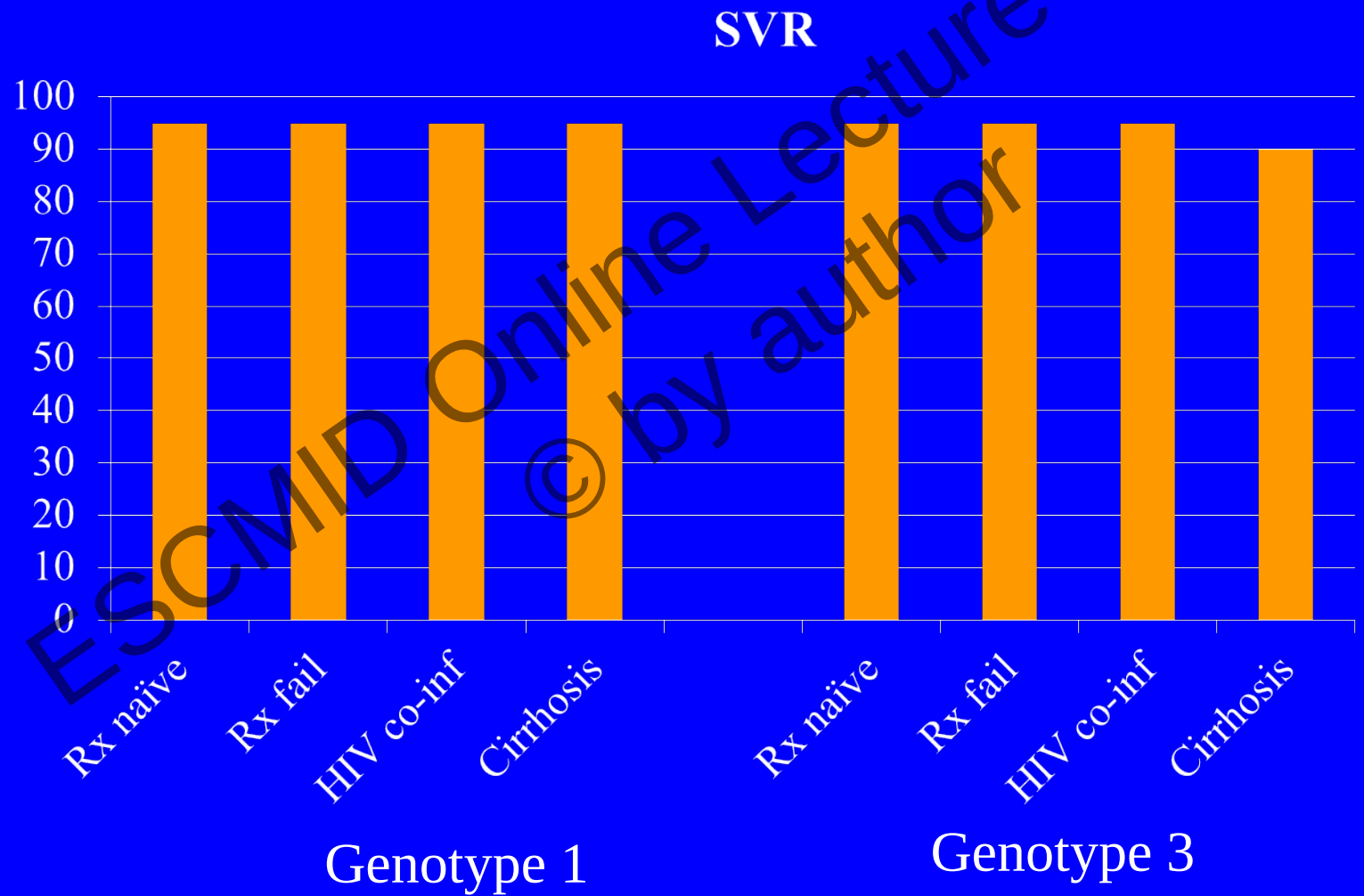


ION-1 Study Design: a randomised, open-label trial evaluating 12 and 24 weeks of treatment with HARVONI with or without RBV in treatment-naïve subjects with HCV GT1[1]

*The on-treatment virological failure patient was confirmed as being non-compliant

REFERENCES: 1. HARVONI Summary of Product Characteristics, November 2014

Perfectovir



Monitoring therapy

- 1999 – IFN/RV, finish course, check for SVR
- 2005 – PEG/RV, apply stopping rule at week 12 for g1 patients
- 2008 – PEG/RV, assess for RGT (week 4), then apply stopping rules
- 2011 – PEG/RV/TPR/BOC – assess for RGT (weeks 4/8, 12/24) + stopping rules
- 2015 – DAAs, finish course, check for SVR