Epidemiology of HCV mixed infection and reinfection in the treatment setting

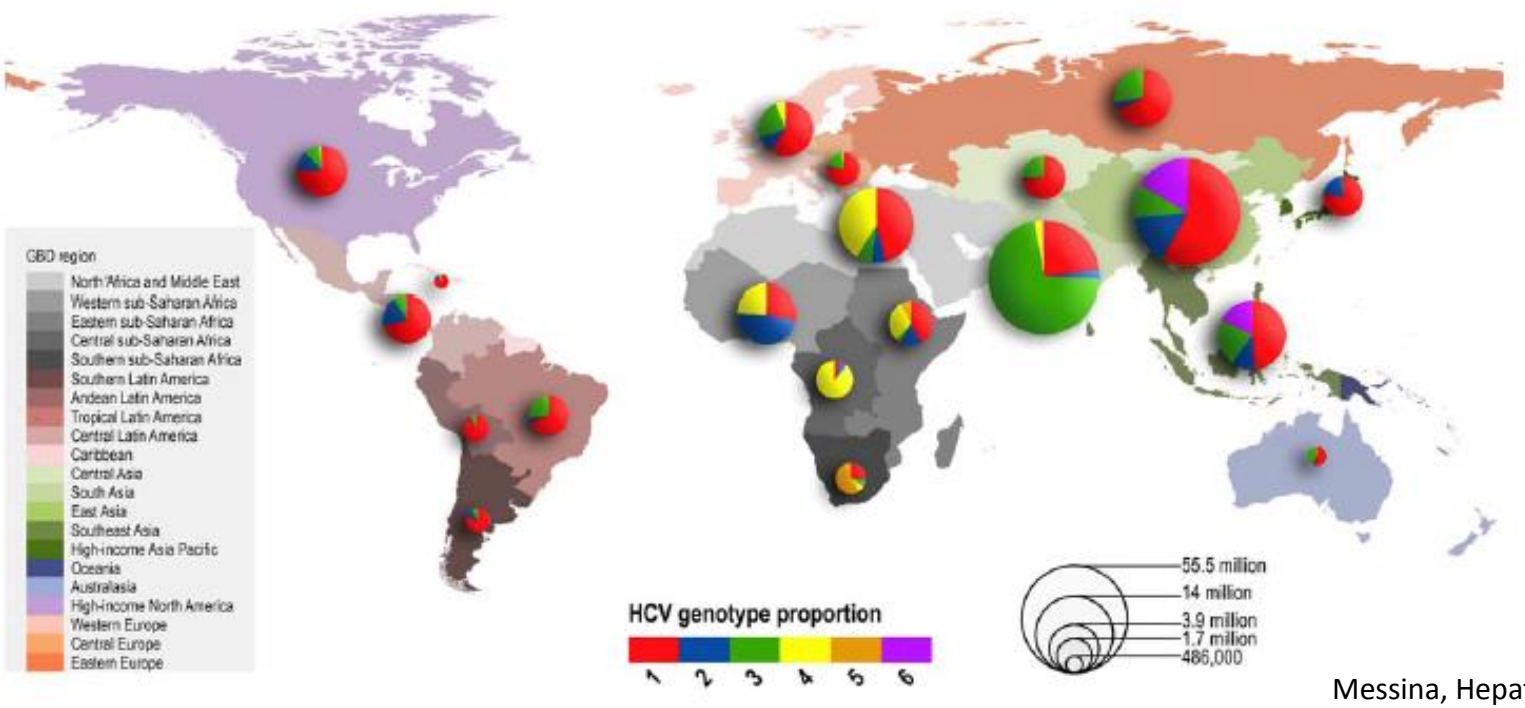
From a virologists' Perspective...

Janke schinkel, MD PhD
Academic Medical Center, Amsterdam
INHSU 2015

Content

- Mixed infection
  - Definition
  - Detection of mixed infection
  - Epidemiology among PWID
- Reinfection
  - Adaptive Immunity to HCV

Mixed infection (1)

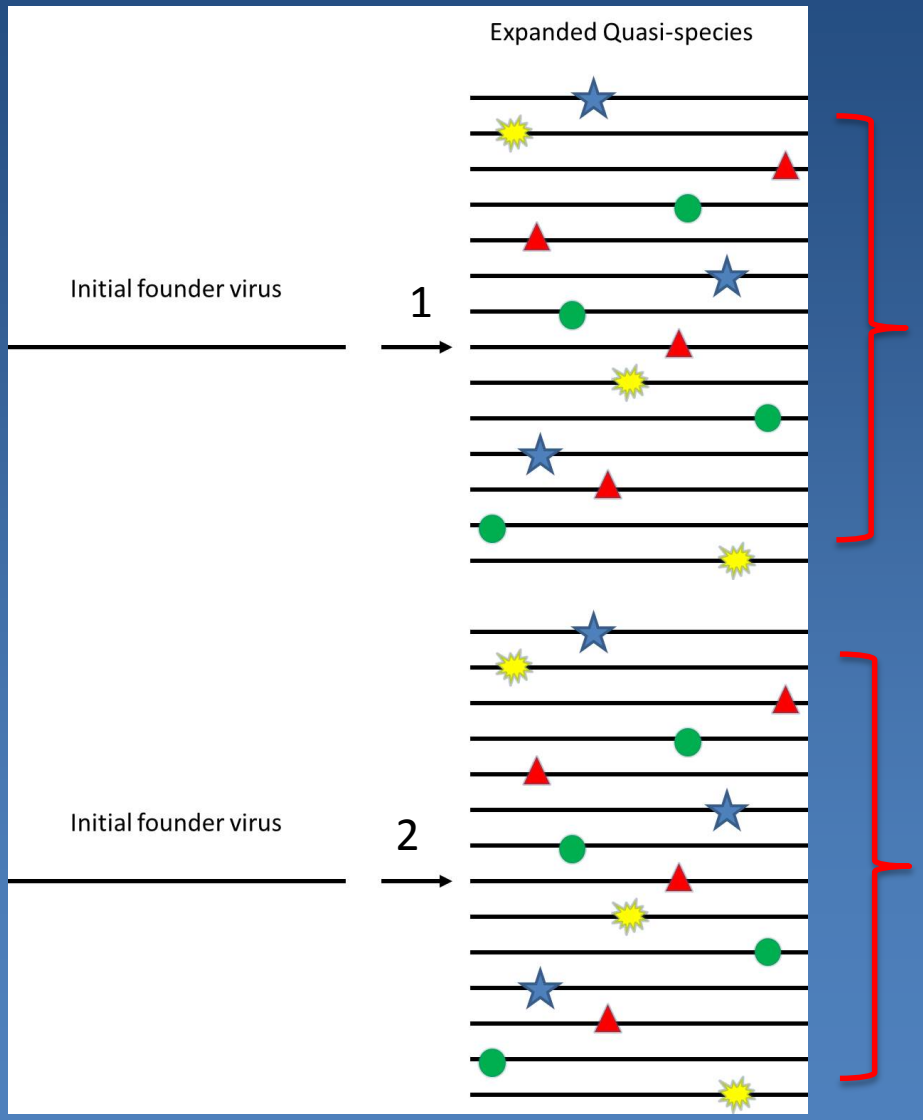


Messina, Hepatology, 2014

DB Smith et al, hepatology 2014

Mixed infection (2)

- Mixed infection
  - Presence of *different variants from the same genotype at the same time*
    - Virus exists within patient many (closely related) different variants (“quasispecies”)
    - Distribution of *pairwise genetic distances* in a mix of viral variants provides the answer
    - *Cut off* needed for genetic distance between variants to distinguish mono- from mixed infection



Genetic distance in mixed infection

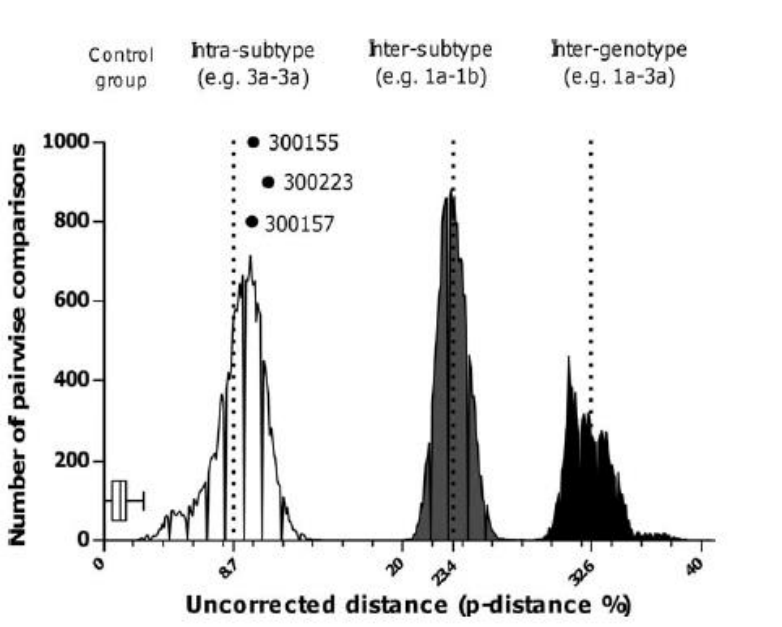
```
Variant A    ACTGACTGA
Variant B    GCTGACTGA
Variant C    ACGGACTGA
```

genetic distance

	variant A	variant B	variant C
variant A	0		
variant B	1	0	
variant C	2	3	0

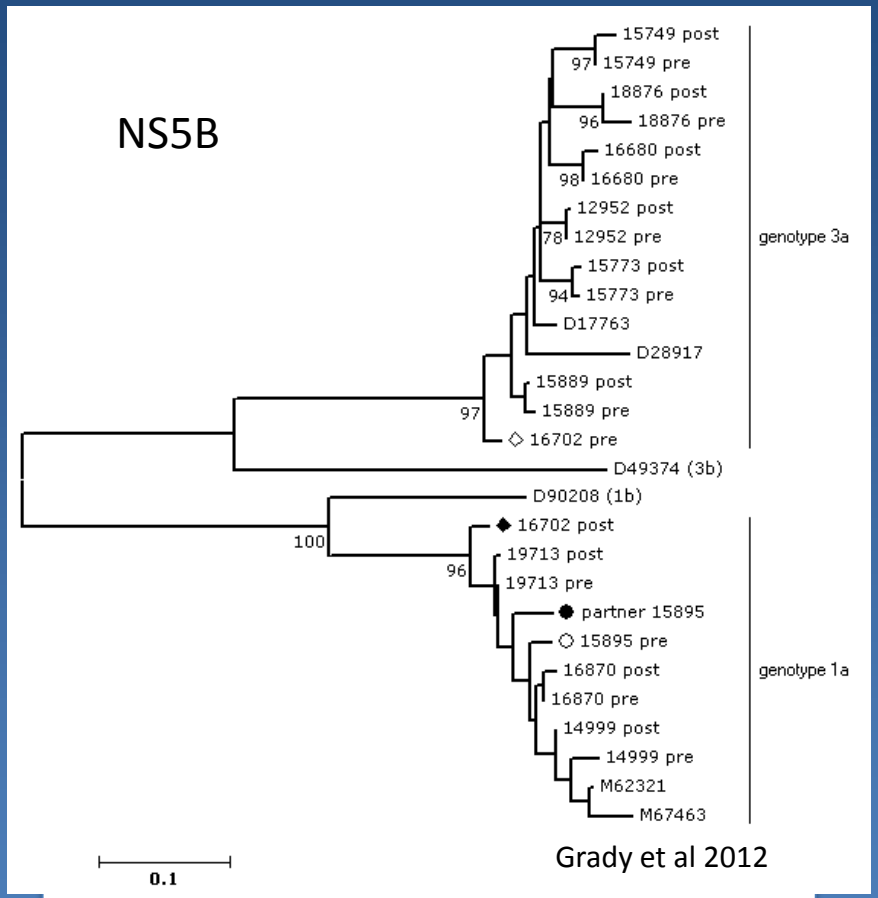
A > C	2
B > C	3
A > B	1

Pham et al, hepatology 2010

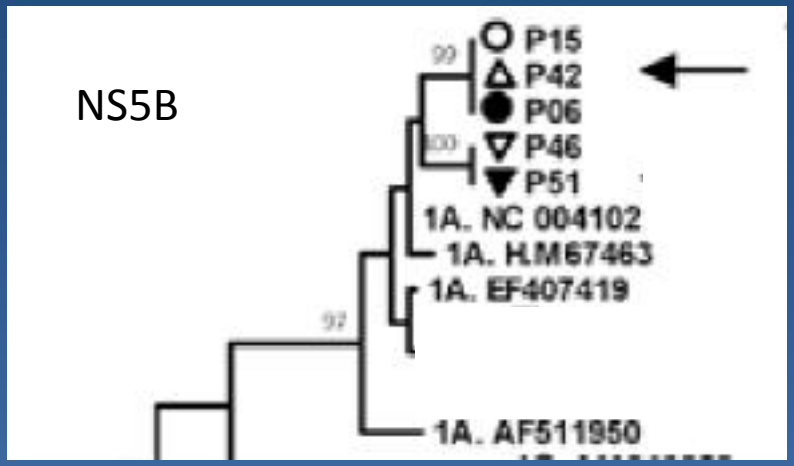


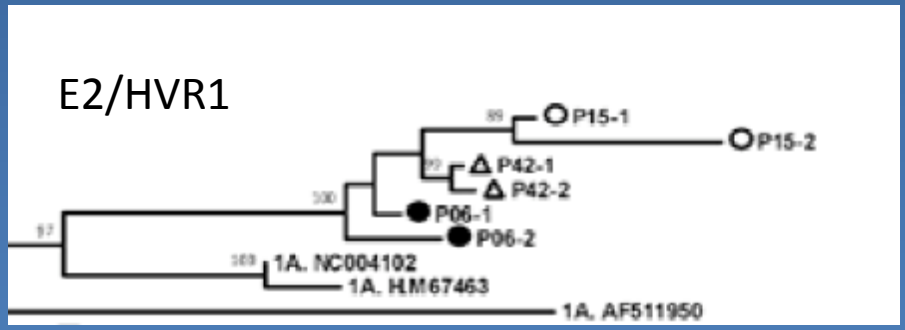
Pairwise genetic distance

HCV genetic variability across the genome



PWID population in Amsterdam





MSM population Amsterdam

Selection of genomic fragment for detection of mixed infection depends on the characteristics of the epidemic

Relevance of detecting mixed infections

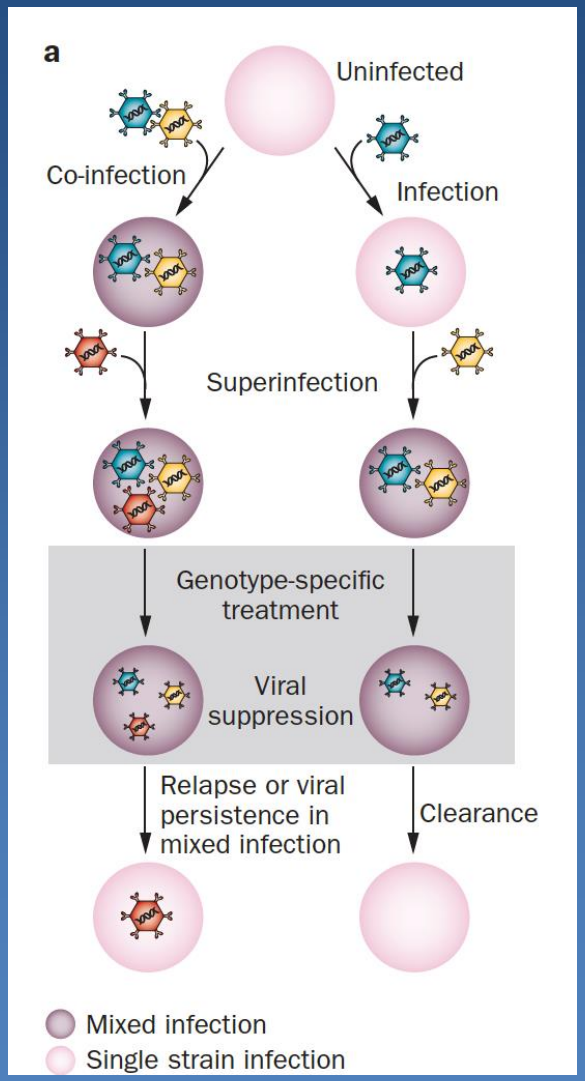
EASL treatment guidelines 2015

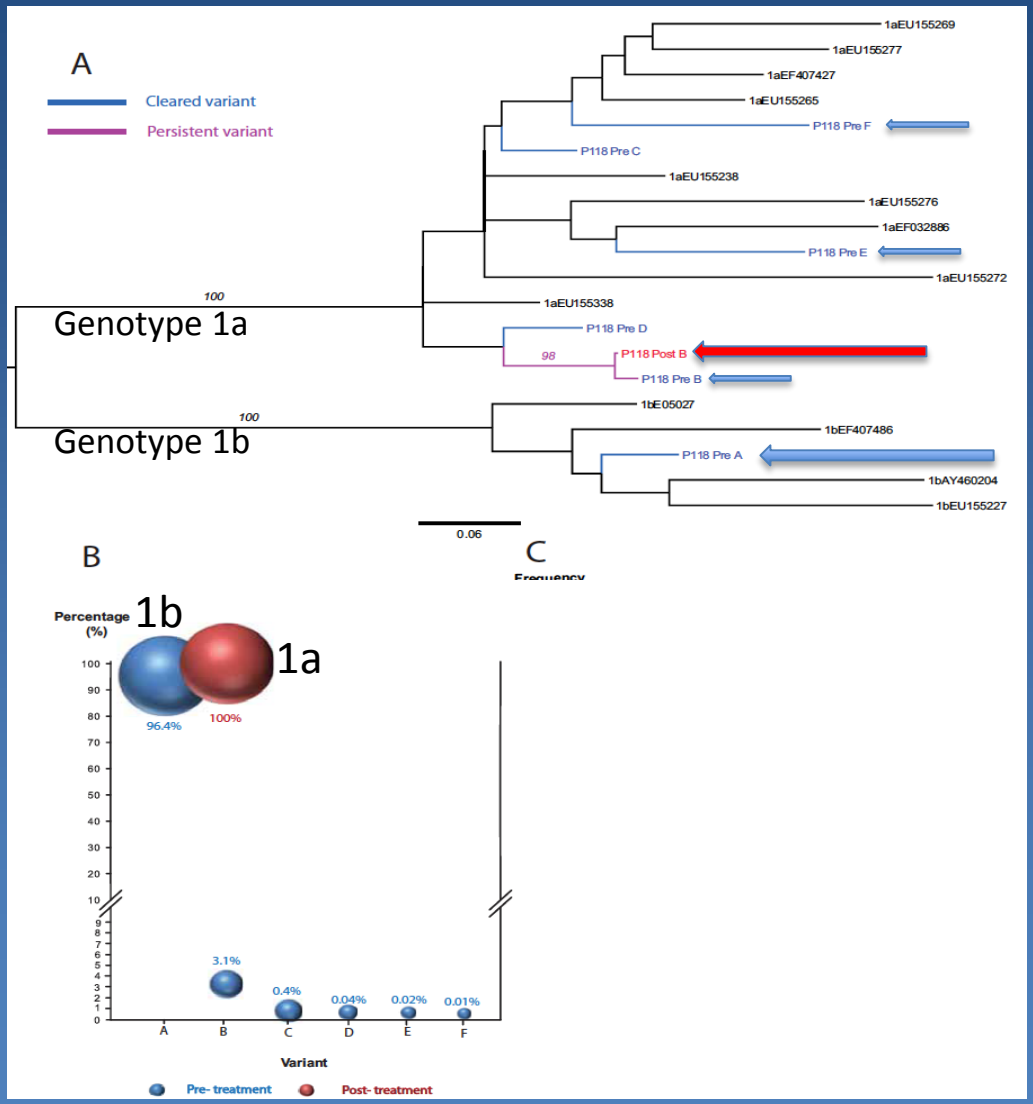
Patients	PegIFN-α, RBV and sofosbuvir	PegIFN-α, RBV and simeprevir	Sofosbuvir and RBV	Sofosbuvir and ledipasvir	Ritonavir-boosted paritaprevir, ombit-asvir and dasabuvir	Ritonavir-boosted paritaprevir, and ombitasvir	Sofosbuvir and simeprevir	Sofosbuvir and daclatasvir
Genotype 1a	12 wk	12 wk, then PegIFN-α and RBV 12 wk (treatment-naïve or relapsers) or 36 wk (partial or null responders)	No	8-12 wk, without RBV	12 wk with RBV	No	12 wk without RBV	12 wk without RBV
Genotype 1b					12 wk without RBV			
Genotype 2	12 wk	No	12 wk	No	No	No	No	12 wk without RBV
Genotype 3	12 wk	No	24 wk	No	No	No	No	12 wk without RBV
Genotype 4	12 wk	12 wk, then PegIFN-α and RBV 12 wk (treatment-naïve or relapsers) or 36 wk (partial or null responders)	No	12 wk without RBV	No	12 wk with RBV	12 wk without RBV	12 wk without RBV
Genotype 5 or 6	12 wk	No	No	12 wk without RBV	No	No	No	12 weeks without RBV

DAA Treatment without interferon is (still) genotype specific

Detection of mixed infections with different subtypes / variants not relevant for treatment

Mixed infections: dynamics of treatment failure





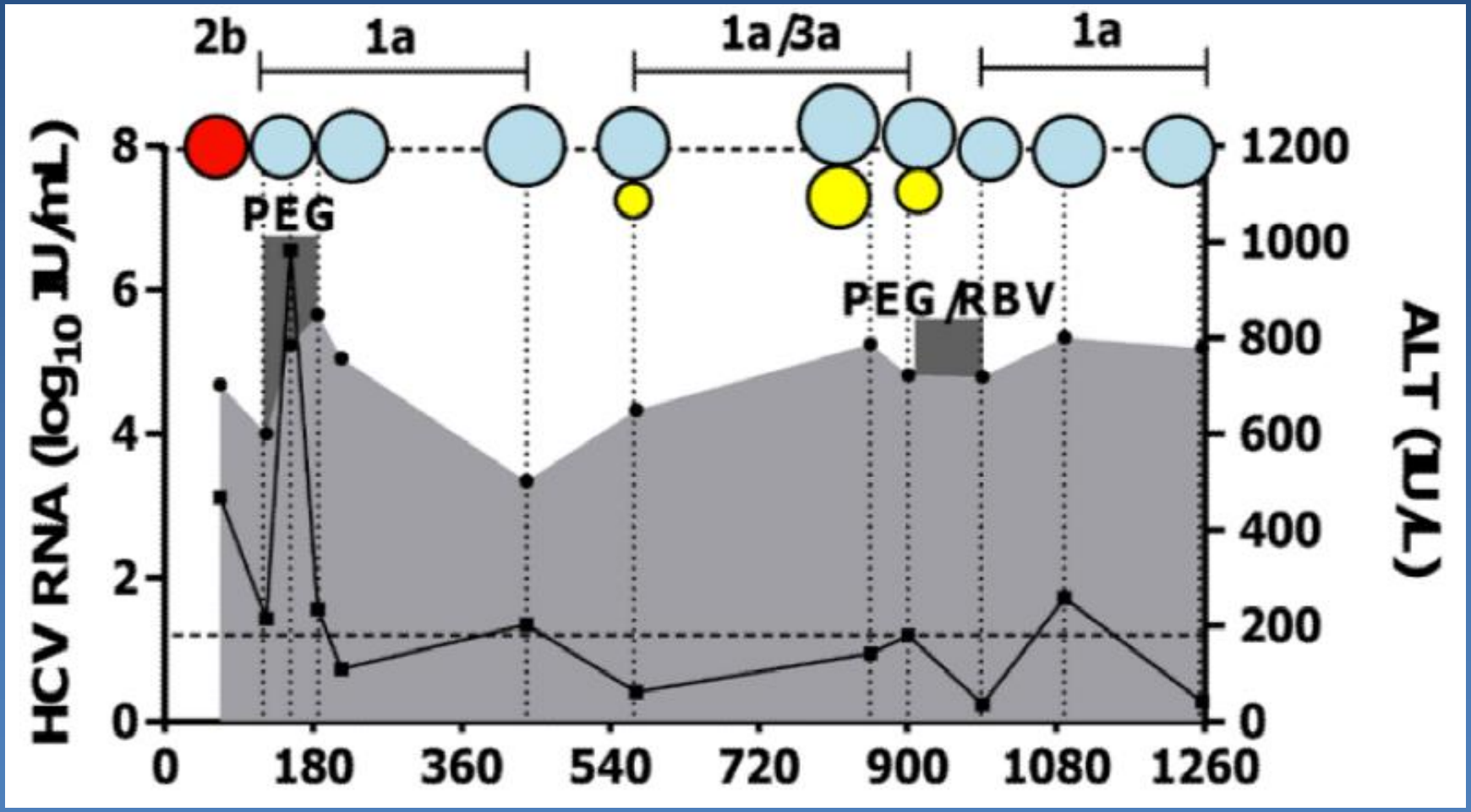
after

before

Cunningham et al, Nature reviews in Gastroenterology and Hepatology, 2015

Adapted from Abdelrahman, Hepatology 2015

multiple infections over time



Grebely, Hepatology et al 2012

How to detect mixed infections wish list

- Sensitive assay
- “Unbiased” PCR
  - Ability to pick up all genotype
- Adequate genotype assignment
- Easy to apply in clinical settings
- Cheap

Methods for detecting mixed infection

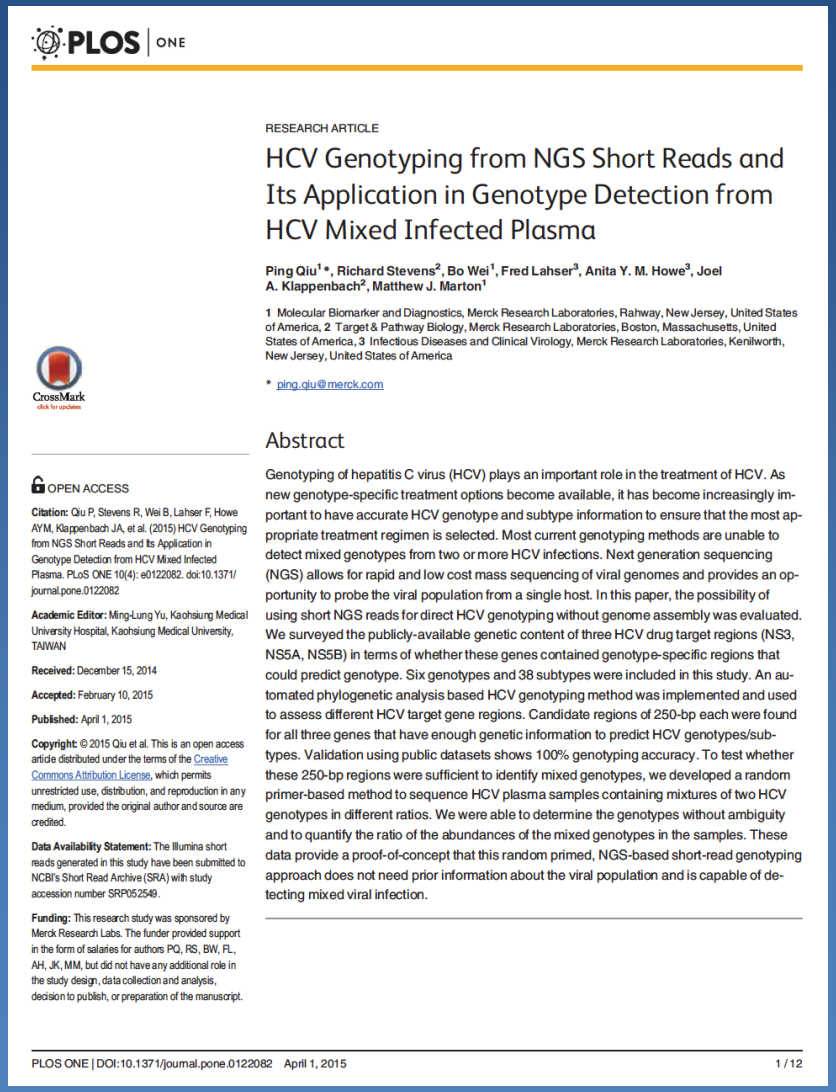
technique	advantages	disadvantages
PCR + Sanger sequencing (core / NS5B)	easy, cheap	Not sensitive, interpretation of mixed bp difficult
PCR, cloning, sequencing	sensitive	More epxensive, time consuming
Genotype specific nested PCR	sensitive	Risk of cross-contamination, time consuming
PCR + NGS	(very) sensitive	No standardized pipeline available yet, expensive

commercial assays for genotyping

assay	technique	genotyping	disadvantages	Performance of detecting mixed infection
Abbott m2000 RealTime HCV Genotype II assay	genotype-specific real-time PCR (specific primer / probes)	1 – 6, Subtype 1a, 1b	Not always resolved (10%)	??, false positive mixed infection reported
Versant HCV genotype assay (LiPA) **2.0**	PCR , hybridisation (5' end, **core**)	Detection of gentoype 1 – 6, subtypes 1a, 1b and some 6	Misclassifies genotype 6 as 1, incomplete assignment,	??, false positive mixed infections

NextGen genotyping

- No PCR, random priming for cDNA synthesis
- Identification of (short) genome fragments for accurate genotyping
- ‘simple’ pipeline without haplotype reconstruction
- Proof of concept: mixed infection (90%/10%) accurately identified



PLOS | ONE

RESEARCH ARTICLE

HCV Genotyping from NGS Short Reads and Its Application in Genotype Detection from HCV Mixed Infected Plasma

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Abstract

Genotyping of hepatitis C virus (HCV) plays an important role in the treatment of HCV. As new genotype-specific treatment options become available, it has become increasingly important to have accurate HCV genotype and subtype information to ensure that the most appropriate treatment regimen is selected. Most current genotyping methods are unable to detect mixed genotypes from two or more HCV infections. Next generation sequencing (NGS) allows for rapid and low cost mass sequencing of viral genomes and provides an opportunity to probe the viral population from a single host. In this paper, the possibility of using short NGS reads for direct HCV genotyping without genome assembly was evaluated. We surveyed the publicly-available genetic content of three HCV drug target regions (NS3, NS5A, NS5B) in terms of whether these genes contained genotype-specific regions that could predict genotype. Six genotypes and 38 subtypes were included in this study. An automated phylogenetic analysis based HCV genotyping method was implemented and used to assess different HCV target gene regions. Candidate regions of 250-bp each were found for all three genes that have enough genetic information to predict HCV genotypes/subtypes. Validation using public datasets shows 100% genotyping accuracy. To test whether these 250-bp regions were sufficient to identify mixed genotypes, we developed a random primer-based method to sequence HCV plasma samples containing mixtures of two HCV genotypes in different ratios. We were able to determine the genotypes without ambiguity and to quantify the ratio of the abundances of the mixed genotypes in the samples. These data provide a proof-of-concept that this random primed, NGS-based short-read genotyping approach does not need prior information about the viral population and is capable of detecting mixed viral infection.

OPEN ACCESS

Citation: Qiu P, Stevens R, Wei B, Lahser F, Howe AYM, Klappenbach JA, et al. (2015) HCV Genotyping from NGS Short Reads and Its Application in Genotype Detection from HCV Mixed Infected Plasma. PLoS ONE 10(4): e0122082. doi:10.1371/journal.pone.0122082

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Data Availability Statement: The Illumina short reads generated in this study have been submitted to NCBI's Short Read Archive (SRA) with study accession number SRP052549.

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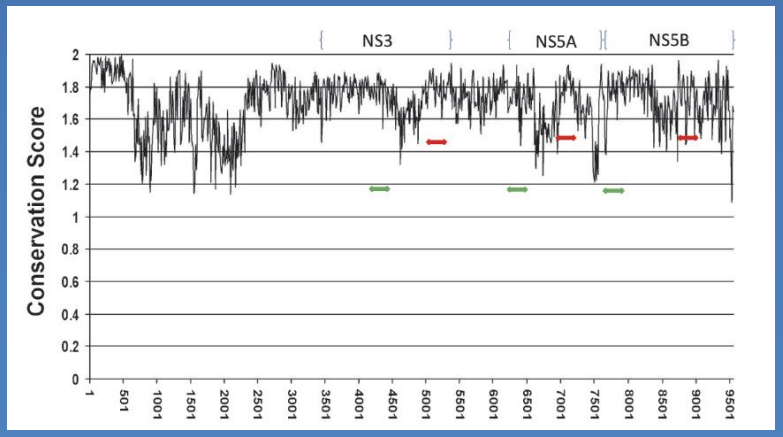
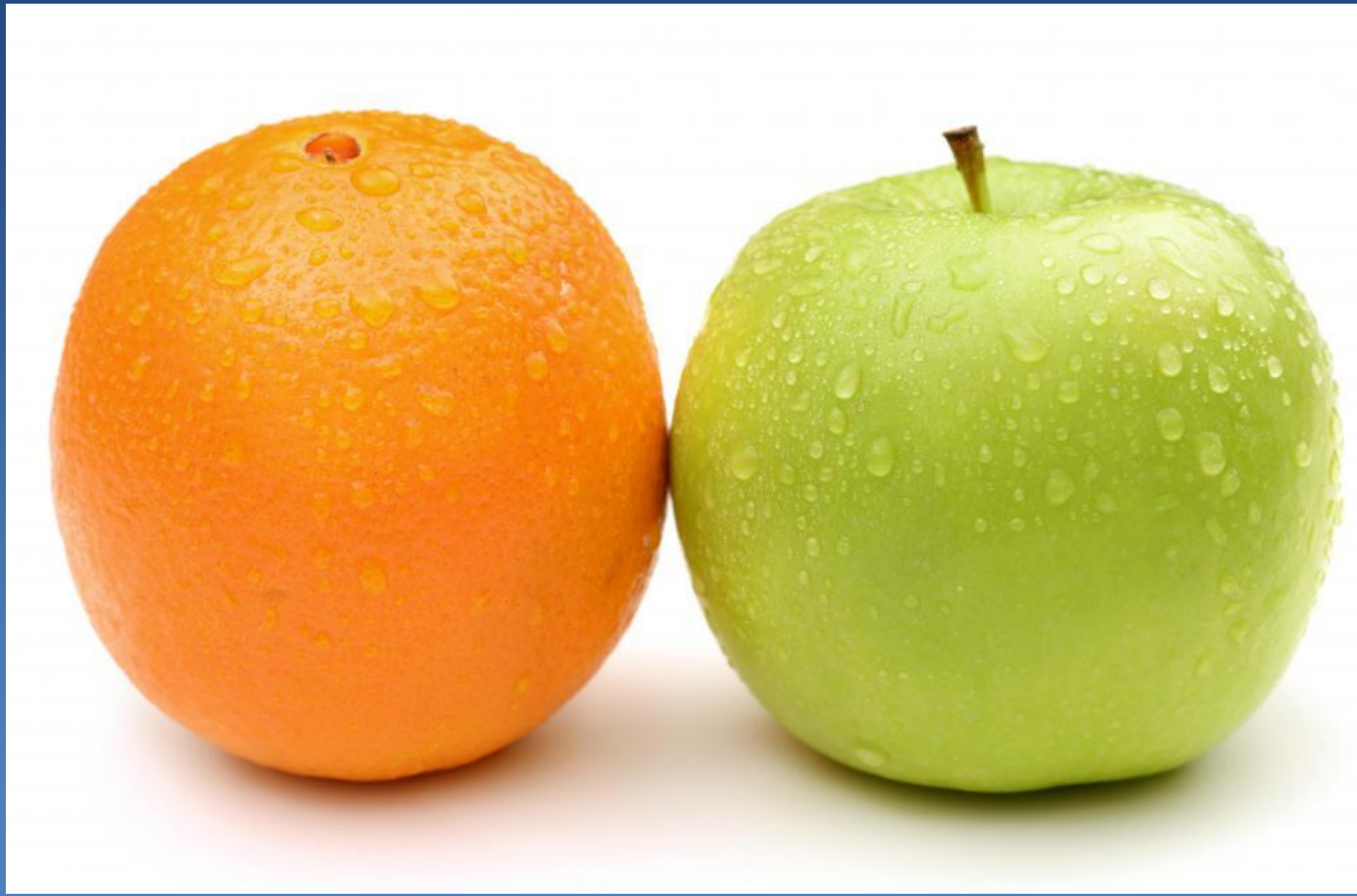


P.Xio et al, Plos One 2015

Epidemiology of mixed infection among PWID

- Observed prevalence depends on
  - Characteristics of population (risk behavior)
  - Persistence of mixed infection
  - Method used

Epidemiology of mixed infections in pWID



Cunningham et al, Nature reviews in Gastroenterology and Hepatology, 2015

The Amsterdam Cohort study

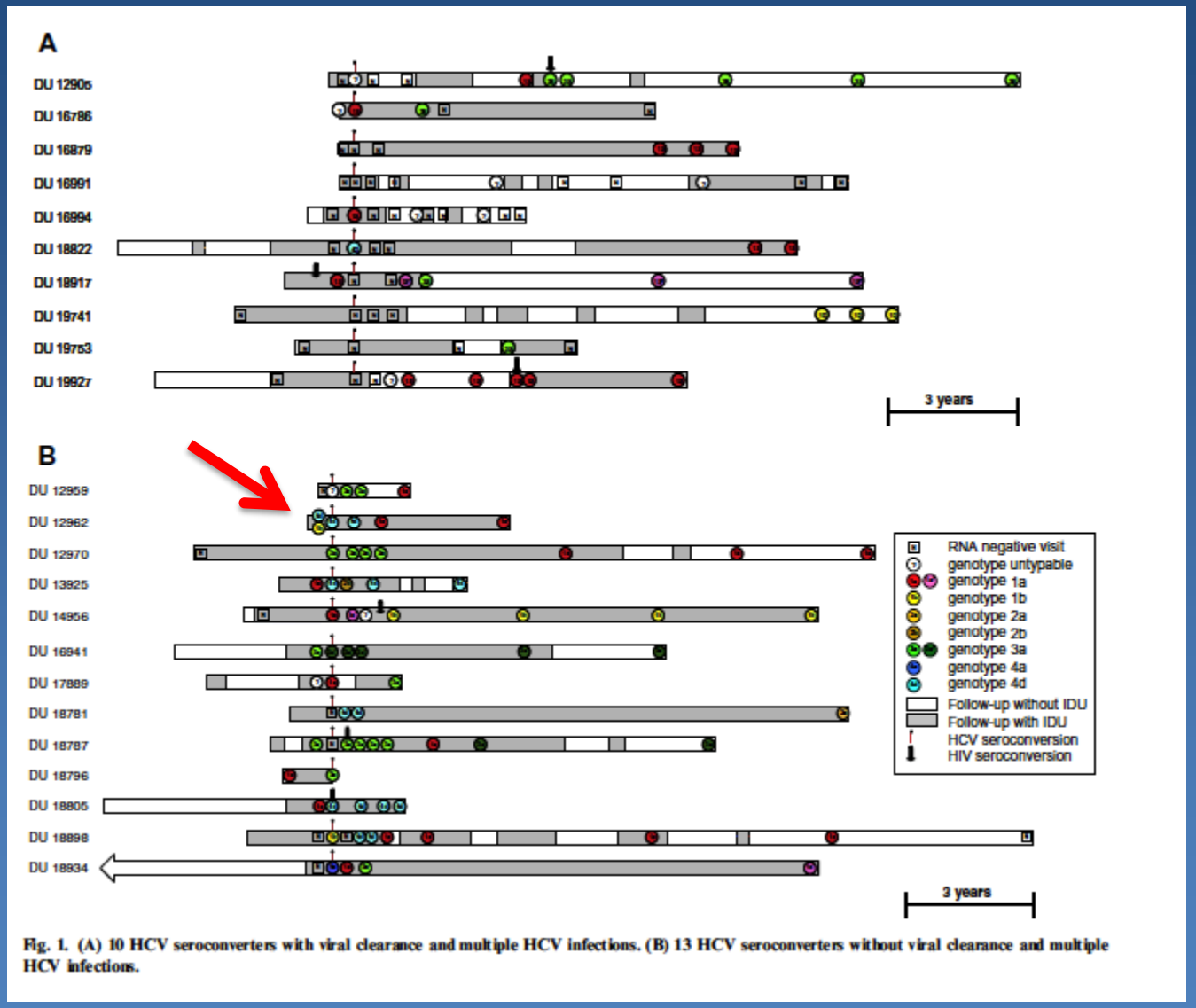
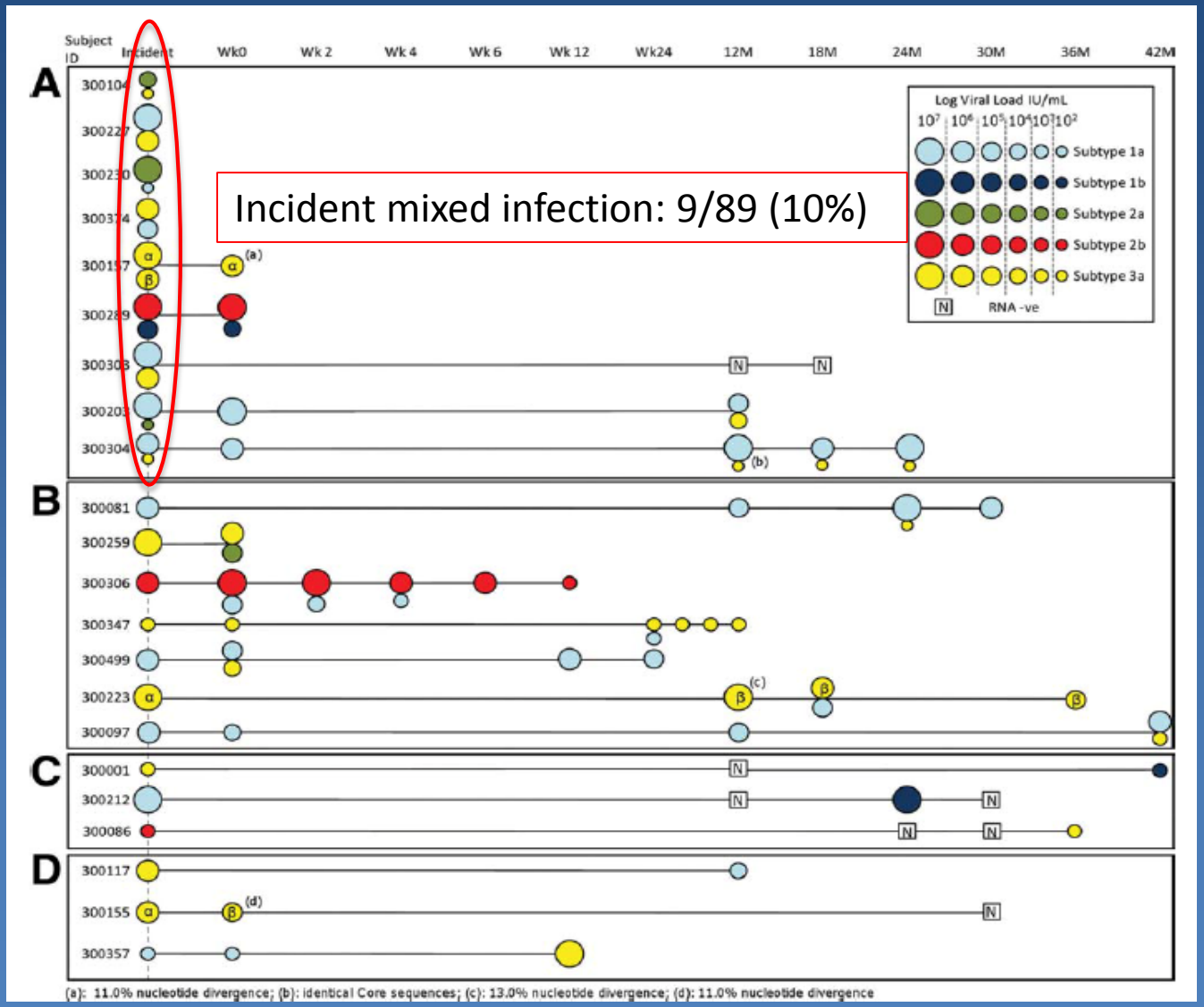


Fig. 1. (A) 10 HCV seroconverters with viral clearance and multiple HCV infections. (B) 13 HCV seroconverters without viral clearance and multiple HCV infections.

Multiple infections in 23/59 (39%) seroconverters

Van de Laar et al J of Hepatology 2009

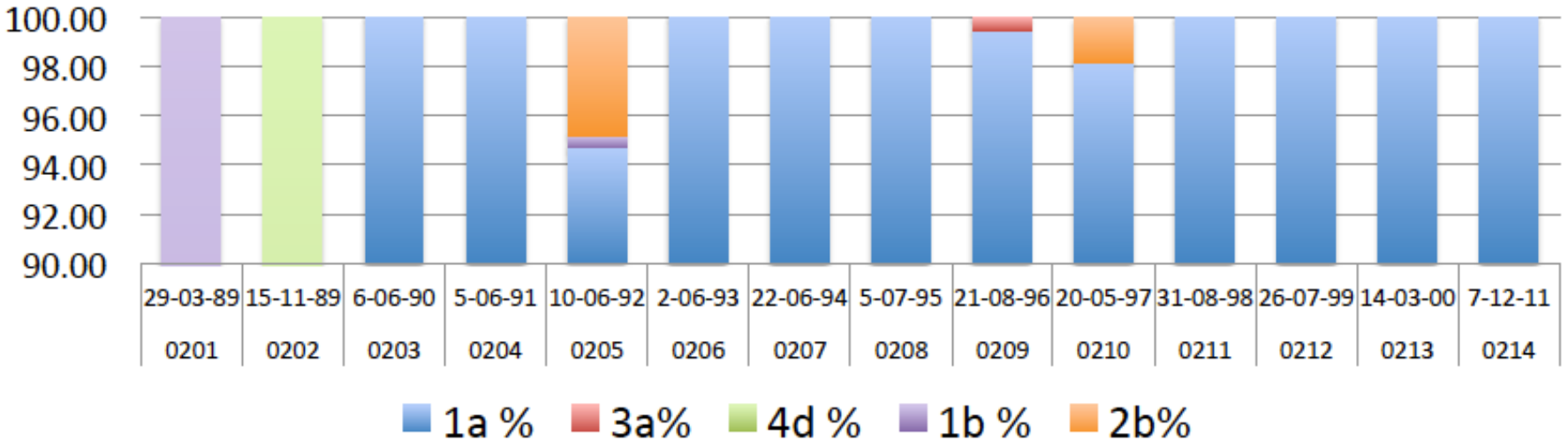
HITS-P cohort



Pham et al, Hepatology 2010

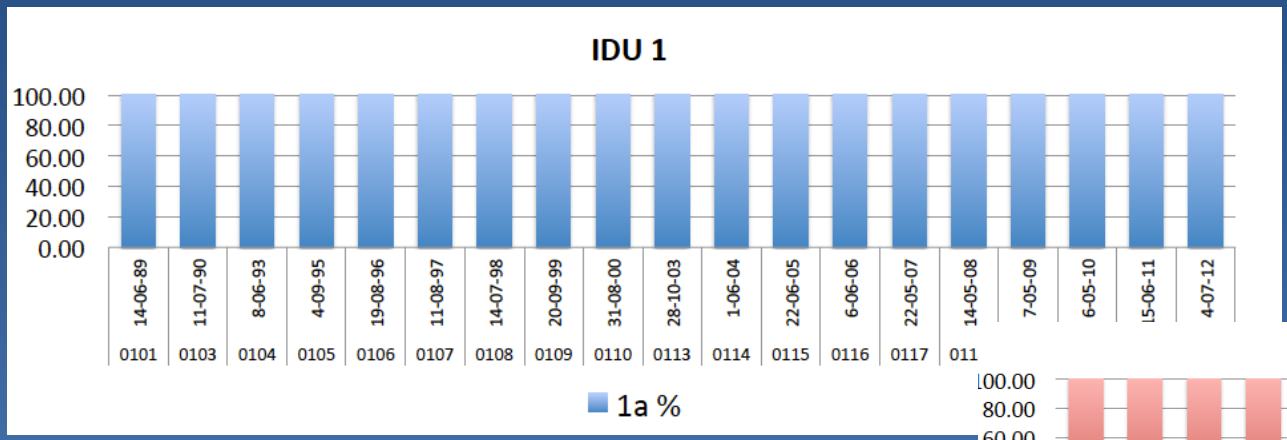
NextGen sequencing

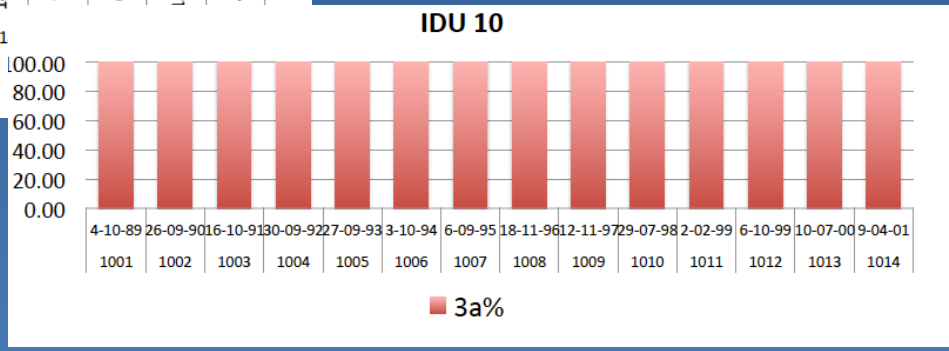
- Amsterdam Cohort Studies among PWID, founded 1985
- 12 participants chronically infected followed from seroconversion
- Median follow up 12 years
- Total follow up: 143 years
- Number of samples: 156, median 13 per subject
- Gene: NS5B fragment (389 bp) according to Murphy et al*. (1 primer pair, second set for genotype 6)

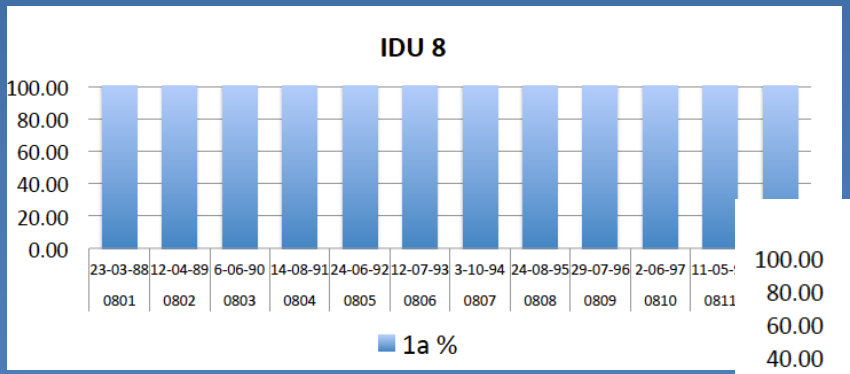


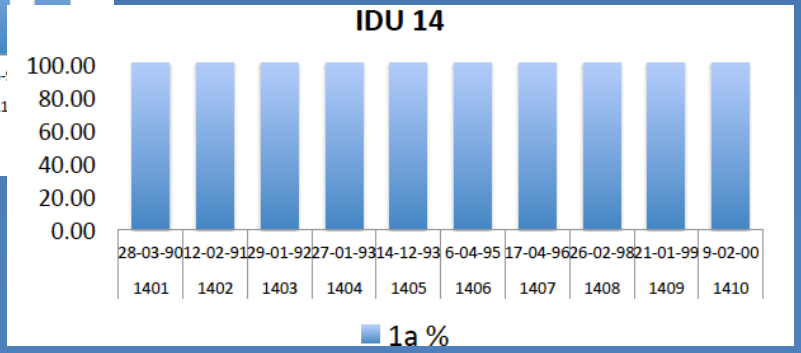
Murphy et a, J Clinical Microbiology 2007

subjects without mixed infections with multiple genotypes (n = 4)

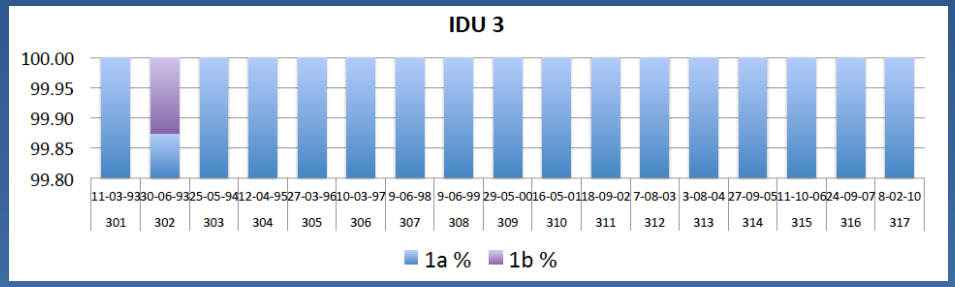


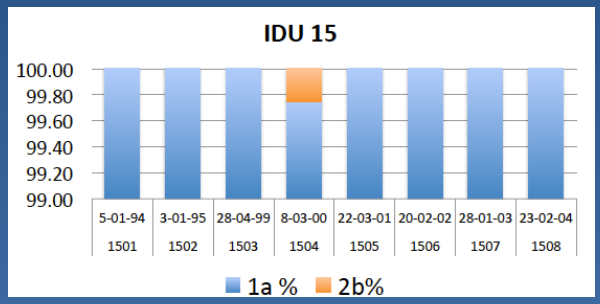


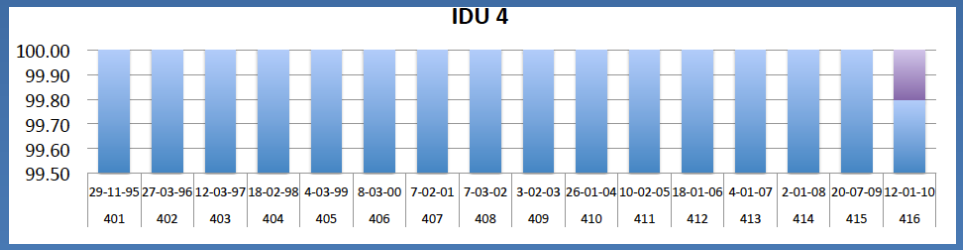


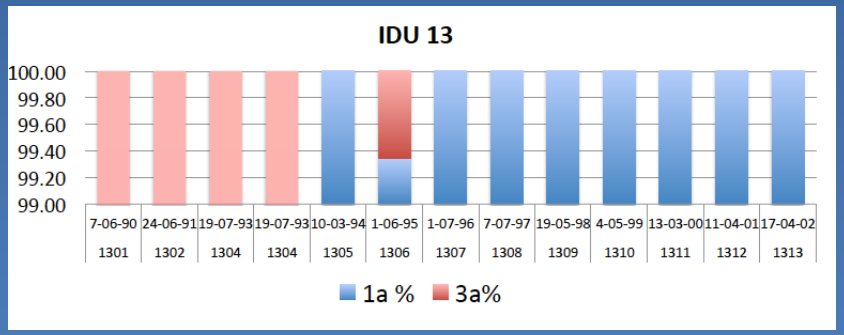


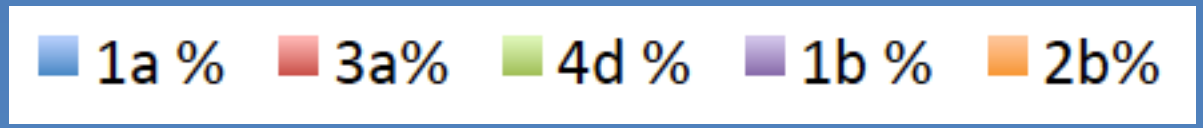
subjects with mixed infections with multiple genotypes, low prevalence of minor variants (< 1%) (n = 4)



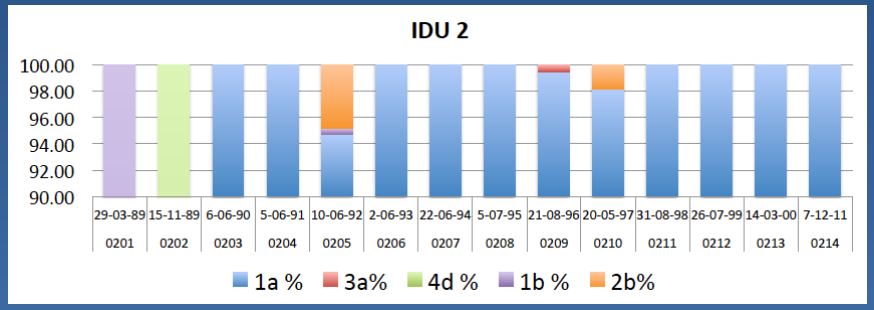


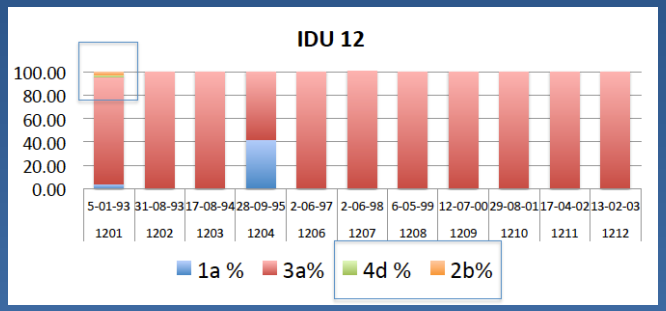


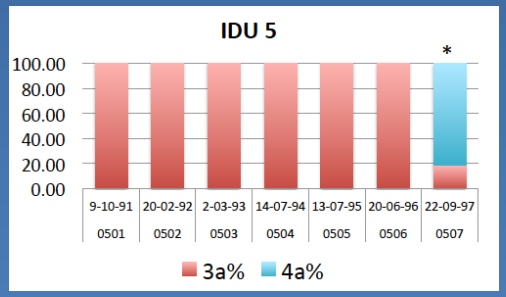


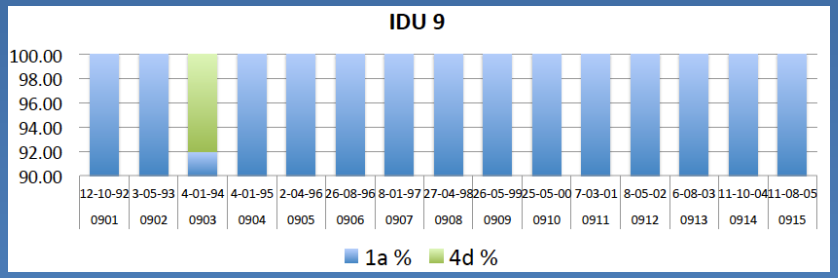


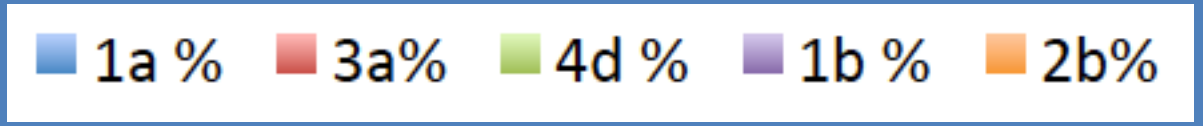
subjects with mixed infections with prevalence of minor variants above 1%(n = 4)











What did we learn ?

- 1/3 of subjects no evidence of mixed infection despite long follow up
- 1/3 of subjects evidence for mixed infection with different genotype present < 1%
- 1/3 of subjects evidence for mixed infection with minor variant > 1%
- ***Mixed infections do not persist***

Quantitave summary of NGS study ACS

N of Persons with multiple consecutive infections	8/12 (67%)
Incidence of superinfections	11/100 PY
N of persons with (ever) a mixed infection	8/12 (67%)
Percentage of samples with mixed infections	7%

Reinfection following SVR in PWID

- Yes…. occurs…

Table 1. Overview of Studies on Hepatitis C Virus Reinfection Following Treatment Among People Who Inject Drugs

Study	Country	Study Design	Geno-typing	Sequence Analysis	No.	Median Age at Treatment Start, y	% Male	IDU Pretreatment <6 mo	IDU Post treatment	Follow-up, Median (IQR)	PY Ever PWID/ PWID Who Continue	No. of Re-infections	Reinfection Rate (95% CI) per 100 PY Ever PWID/PWID Who Continue
Backmund et al, 2004 [8]	Germany	Pros	Yes	No	18	32	61	NA	9	Mean 2.8 (SD 0.8–5.1)	50.8/23.8	2	3.94 (0.48–14.22)/ 8.4 (1.02–30.36)
Dalgard et al, 2002 [11]	Norway	Pros	Yes	No	27	30	66	0	9	5.4 (1.1–6.8)	125.0/40.0	1	0.8 (0–5)/2.5 (0–14)
Currie et al, 2008 [10]	US	Pros	No	No	9	46 (mean)	88	NA	2	3.6 (3.2–6.0)	38.0/3.5	1	2.63 (0.07–14.66) 28.57 (0.72–159.19)
Grebely et al, 2010 [13]	Canada	Pros	Yes	Yes	35	44 (mean)	86	19	16	2.0 (0.4–5.0)	62.5/37.7	2	3.20 (0.39–11.56)/ 5.30 (0.64–19.16)
Bate et al, 2010 [9]	Australia	Pros	Yes	No	57	NA	NA	NA	NA	NA	NA	5	NA
Grady et al, 2012 [12]	Netherlands	Pros	Yes	Yes	42	51	73	5[a]	11	2.5 (1.6–3.7)[b]	131.6/32.3	1	0.76 (0.04–3.73)/ 3.42 (0.17–16.90)
Grebely, 2012 [14]	Australia	Pros	Yes	Yes	88	36	72	33[a]	NA	1.2 (0.1–3.0)[b]	108	5	4.7 (1.9–11.2)

Abbreviations: CI, confidence interval; IDU, injection drug use; IQR, interquartile range; NA, specific information was not available; Pros, prospective; PWID, people who inject drugs; PY, person-years.

[a] During treatment.

[b] Follow-up from end of treatment.

Grady et al, CID, 2013

But…

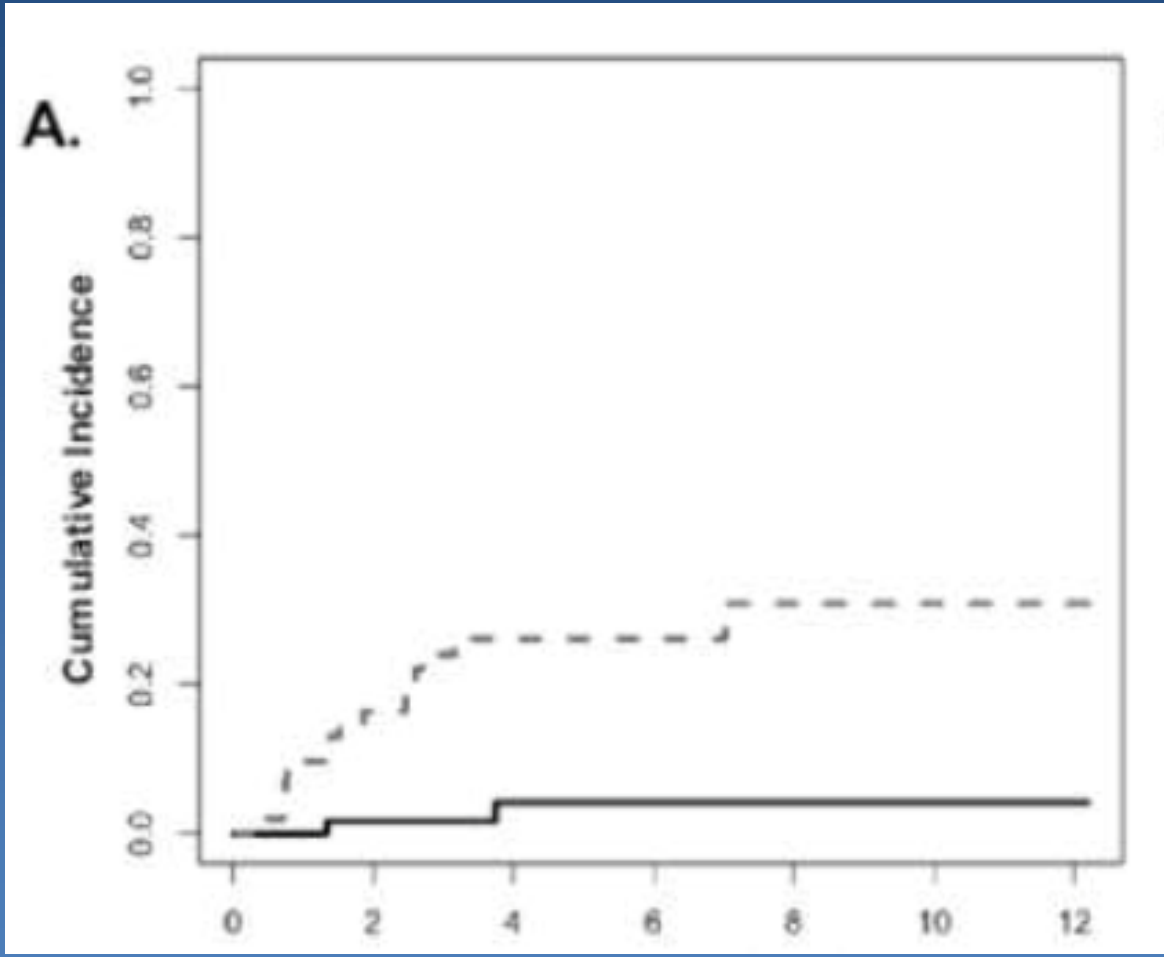
Secondary infection following spontaneous clearance have:

- Higher clearance rates
- Lower peak viremia
- Shorter duration of viremia upon reclearance

(Osburn 2010, Sacks-Davis 2015)

Adaptive immune responses are generated following spontaneous clearance of primary infection

Incidence of reinfection following SVR in HIV+ MSM with acute HCV



Reinfection with different genotype

Reinfection with same genotype

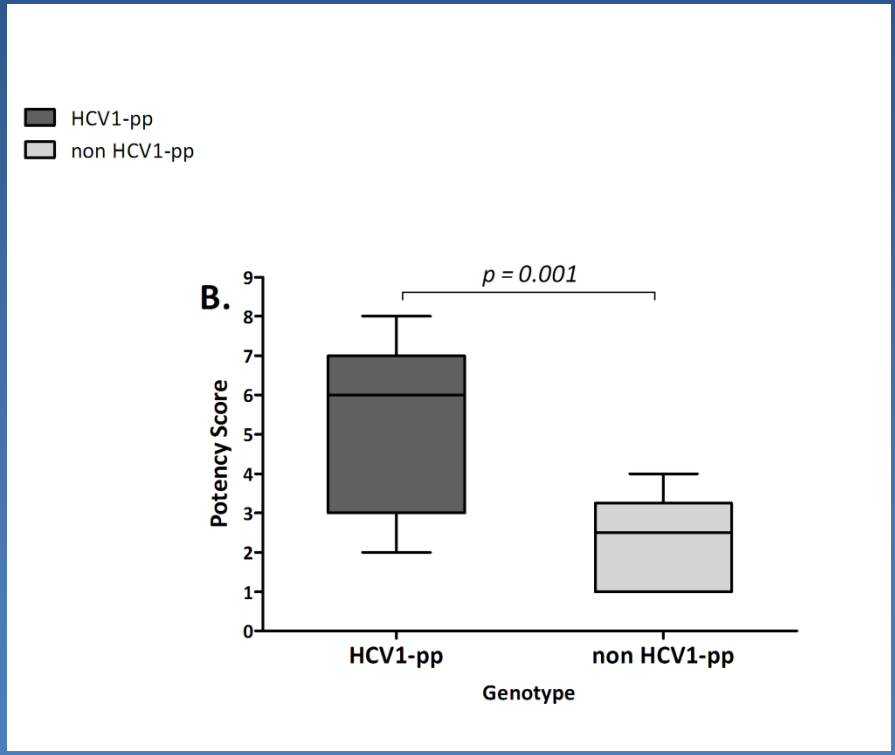
Adaptive responses genotype specific following treatment induced clearance?

Thomas et al, AIDS 2015

Role of neutralizing antibodies

Functional study

- HIV + MSM (MOSAIC)
- Treatment induced clearance of acute HCV-1a infection
- Neutralizing responses in sera were more potent against genotype 1a viruses
- Protection against subsequent HCV-1a infections following SVR



Thomas et al, submitted

conclusions

- Mixed infections occur frequently among PWID (7% mixed infections in ACS, 10% in HITS-p..)
- They tend not to persist
- They are therefore not an obstacle for current treatment regimens
- Reinfection do occur among PWID following SVR with a reported incidence 1 – 8 per 100 PY
- (partial) protective immune responses are generated, even in the HIV-infected population
- Allow more time before treating a secondary infection?
- More data are needed on outcome of DAA-treatment in PWID, risk of reinfection, and the likelihood of spontaneous clearance of reinfections

Thanks to..

- Participants of the ACS and MOSAIC
- My co-workers at the AMC and the Public Health Service in Amsterdam
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