The Emergence of New Viral Strains Following Treatment Failure in an HIV-Positive Cohort Infected with Acute HCV

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Background

More than 1000 cases of acute hepatitis C (HCV) in HIV-positive men-who-have-sex-with-men (MSM) have been reported in urban centres in the Europe, Australia and the USA.[1,2,3]

Following treatment, sustained virological response rates (SVR) in acutely HCV/HIV co-infected patients are lower than in acutely HCV mono-infected individuals (59 versus 98%), [1,4] but the reasons for this are not understood.

In HCV-infected patients, the virus circulates as a mixture of closely related but distinct genomes called quasispecies. We studied the dynamics of quasispecies in pre- and post-treatment samples taken from patients who failed standard of care therapy (48 weeks of pegylated interferon alpha and ribavirin) in a chronic HIV/acute HCV cohort of 160 patients.[5]

Methods

A group of 16 patients failed to respond to treatment. A 220 bp region of the E2 envelope gene including the hypervariable region 1 (HVR-1)(Fig.1) was amplified using nested RT-PCR using a combination of genotype-specific fusion primers.(Fig 2)

PCR products were sequenced by direct sequencing (DS), clonal analysis (CA) and next generation sequencing using a pyrosequencing approach (NGS). Phylogenetic trees were constructed using the maximum likelihood (ML) method.

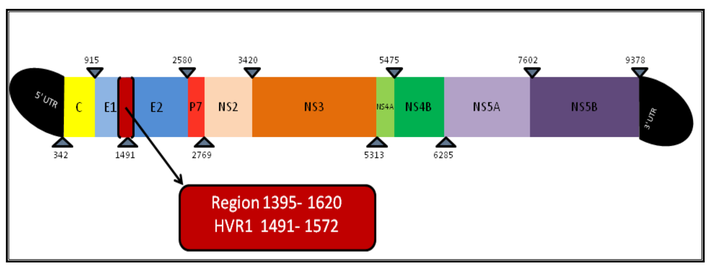


Fig. 1 Schematic representation of HCV genome

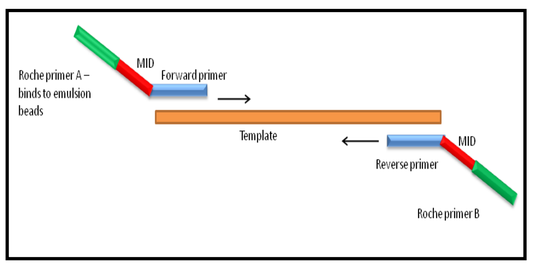


Fig. 2 Fusion primer

Results

Using DS, in the 16 patients that failed treatment (six relapsers, six null responders and four partial responders), 60% of patients had evidence of a "new variant" post-treatment. However, CA and NGS results revealed that 66% of such "new variants" were present in pre-treatment samples, representing new dominance of a pre-existing minority strain that was not detected by DS. Only two patients had completely new strains, which were presumed to represent re-infection. (Table 1, Fig 3, Fig 4)

NGS was superior to CA in detecting the dominance of pre-existing minority strains in 25% of patients. Both techniques detected multiple strains in 50% of patients that were missed by routine diagnostic methods (DS).

Conclusion

In HCV treatment failure, the emergence of new viral strains may most commonly be attributed to new dominance of pre-existing minority variants rather than re-infection.

Hypotheses

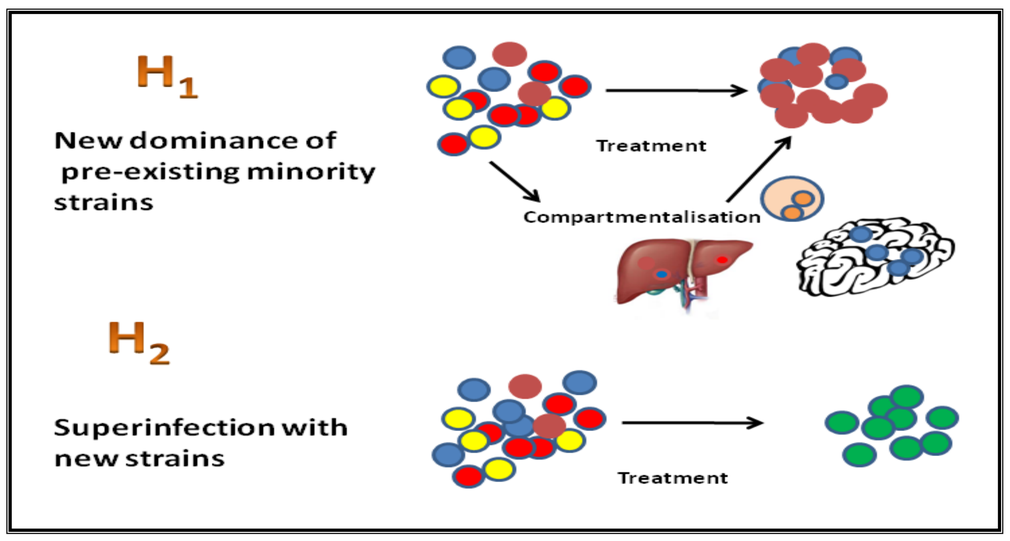


Table 1

Study No	Pattern	Number of variants Pre	Post	Pattern
P112	Null response	3	3	Persistent Infection
P38	Null response	3	2	New Dominance
P81	Null response	3	2	Superfinfection
P67	Null response	1	1	Persistent Infection
P63	Null response	3	3	Superinfection
P118	Null response	2	1	New Dominance
P141	Relapse	3	1	New Dominance
P75	Relapse	2	2	Persistent Infection
P55	Relapse	1	2	New Infection
P101	Relapse	1	1	Persistent Infection
P76	Relapse	3	1	New Dominance
P131	Relapse	1	3	Superinfection
P21	Partial response	2	3	Superinfection
P31	Partial response	4	2	Persistent Infection
P57	Partial response	2	1	New Dominance
P105	Partial response	1	2	New infection

Fig 3. New Dominance

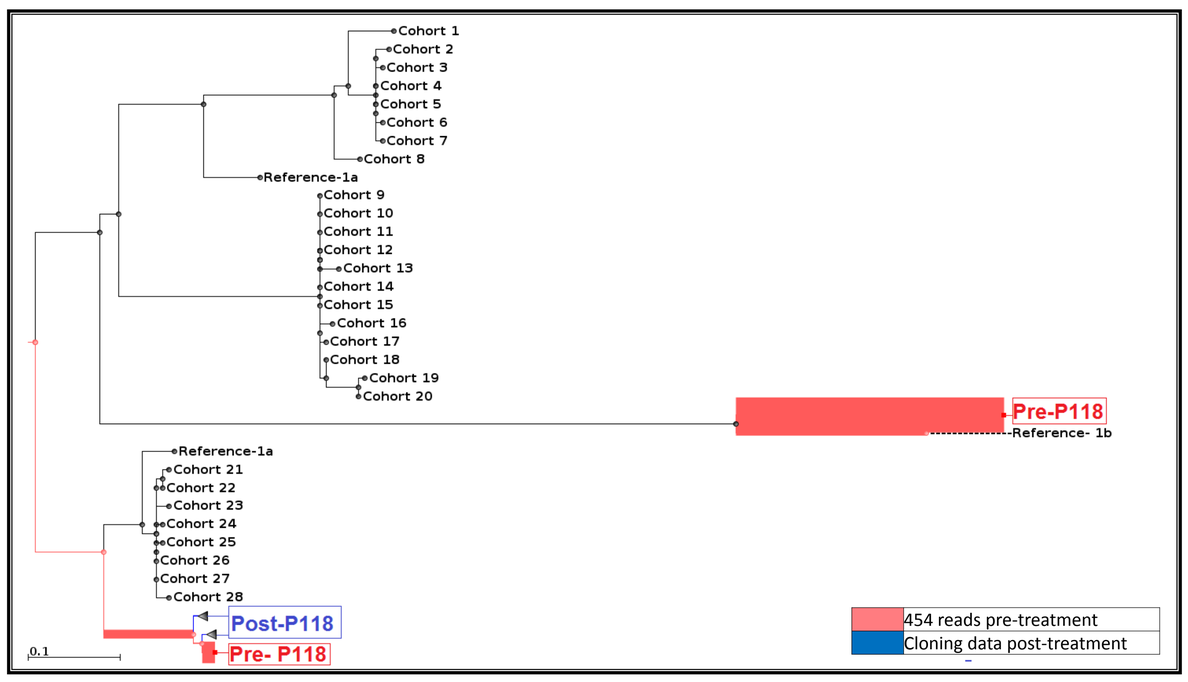
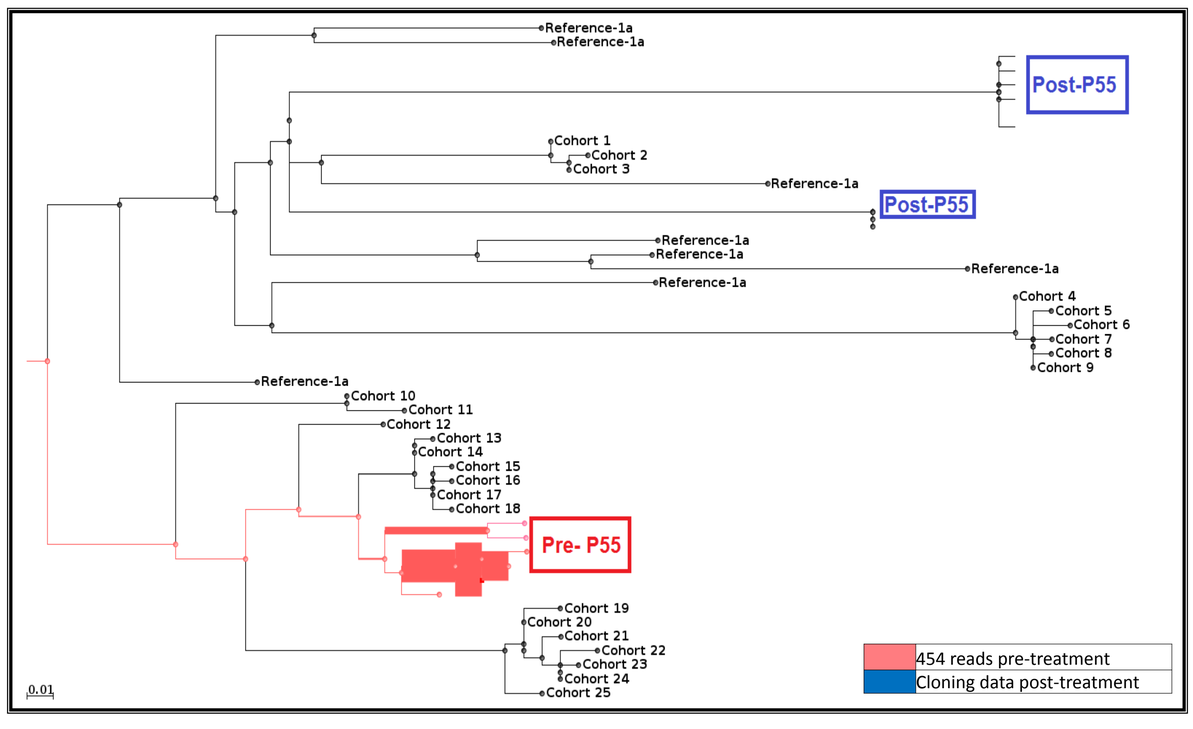


Fig 4. New Variant



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