Resistance-associated Mutations and Genotypes of Hepatitis C Virus NS5B Region in Individuals naive to Sofosbuvir Treatment in Cameroon

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Introduction: HCV Epidemiology (WHO 2017)

• Global estimate of 71 million people have chronic hepatitis C infection

• Blood borne and vertical transmission (upto 50%)

• Approximately 399 000 people die each year from hepatitis C

• Antiviral drugs can cure more than 95% of persons with hepatitis C infection

• Access to diagnosis and treatment is low in LMIC

• There is currently no vaccine for hepatitis C
Introduction: HCV Genetic Diversity (I)

Hepaciviridae genus and Flaviviridae family
9.6kb genome
Phylogeny of HCV Genotypes

1) High rate of evolution and its long-term association with human populations (Smith DB, 1997)
3) Seven known genotypes (Genotype 1 to Genotype 7) and over 67 subtypes (Murphy et al, 2015)
4) HCV recombinant CRF01_1b2k: only known HCV recombinant in widespread circulation (Leitner T, 2005)
5) HCV Genotype 7 described in 2015 (Murphy et al, 2015)
HCV Life cycle

Long ORF of polyprotein of about 3000 amino acids
Polyprotein is cleaved to at least 10 polyproteins by cellular and viral proteases
Polyprotein RNA-dependent-RNA polymerase encoded by NS5B gene
HCV RNA-dependent-RNA polymerase (RdRp)

• The **HCV RdRp** contains six highly conserved motifs designated A-F, just like all known RdRps (Poch et al, 1989)

• The **HCV NS5B** encodes the catalytic subunit and is central for HCV RNA replication

• The **HCV RdRp** is the last viral protein to be translated from the HCV genome

• The **HCV RdRp** remains a target of choice for both nucleoside and non nucleoside inhibitors (Beaulieu and Tsantrizoa, 2004; Wu and Hong, 2003)

• The **RdRp** is an action site for NS5B inhibitors such as SOFOSBUVIR (SOF), a nucleoside polymerase inhibitor.

[Structure of the HCV RdRp (Lesburg et al, 1999)]
HCV Proteins as Drug Targets

Prockos et al, 2017
HCV Seroprevalence & Molecular Epidemiology in Cameroon

HCV Seroprevalence

• Disparities according to region and age of study population
• General population is 6.4%
• General population of age above 50 years is 9%
• 3.7% to 20.4% in 9 administrative regions in Cameroon (Njouom et al, 2015)

HCV genetic diversity

• 252 NS5B sequences: Genotype 1, genotype 2, Genotype 4 of sequences reported from 1996 to 2013 in LANL (Torimiro et al, 2016)
• 220 NS5B sequences: Genotype 1 (5.7%), genotype 2 (2.2%) and Genotype 4 (91.9%) (Njouom et al, 2012)
• Several HCV subtypes with some unclassified variants (Li et al, 2013)
• HCV recombinant G4/G1 (Illes et al, 2015)
• HCV Genotype 1 possible origin in Cameroon (Li et al, 2013)
HCV Genotyping by Phylogeny

HCV sequences reported in the LANL from 1996 to 2013 (Torimiro et al, 2016)

HCV NS5B phylogeny

HCV E1 phylogeny

HCV Core phylogeny
Aim of study

- Rapidly changing epidemiology of HCV
- Direct acting agents (DAAs) introduced in 2011
- NS5B inhibitors – interrupt the action of RdRp
- New Treatment guidelines for Cameroon in 2016 from pegIFN/RBV to SOFOSBUVIR-containing combination

*Baseline transmitted drug resistance for HCV at the population level*

**Preparedness to detect:** drug resistant HCV variants and develop an early warning system (public health approach)

**Preparedness to respond:** treat those who are infected with resistant HCV variants and minimize transmission of resistant variants
Study design

- **Study sites**: Hepatitis Treatment Centres in Douala and Yaounde
- **Study population**: 54 adults of age 31 to 81 years
- **Study period**: November 2016 to November 2017
- **Direct sequencing** of HCV NS5B using the 3130XL DNA analyzer (ABI)
- **Prediction of resistance associated mutations** to SOF: Geno2Pheno tool version 0.92
- **Genotyping** by phylogeny
Results/Discussion:
HCV Genotypes and Subtypes

Phylogenetic Tree of HCV NS5B

HCV Genotypes 1, 2 and 4
HCV subtypes: 4f, 4p, 4a, 4t, 1e, 1h, 1l
Results/Discussion: HCV Genotypic Drug Resistance

S282T
- 0.4%: Torimiro et al (2016)
- 0%: Costantino et al (2015)

N316C
- 12.9%
- 43%: Costantino et al (2015)

KEY
- Orange: Primary mutation
- Light blue: Polymorphism

S282L = 1.9%
M/C289R = 1.9%
C289F/M = 7.4%
# Resistance associated Mutations in HCV NS5B in SOFOSBUVIR-naïve Patients in Cameroon

<table>
<thead>
<tr>
<th>Drug</th>
<th>NS5B mutation</th>
<th>Resistance Prediction</th>
<th>Frequency (%)</th>
<th>Other comments</th>
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<tbody>
<tr>
<td>Sofosbuvir (SOF)</td>
<td>S282T</td>
<td>resistance</td>
<td>0.4</td>
<td>Primary mutation selected for SOF resistance</td>
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<tr>
<td>Sofosbuvir (SOF)</td>
<td>S282C</td>
<td>not known</td>
<td>0.4</td>
<td>Polymorphism</td>
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<tr>
<td>Sofosbuvir (SOF)</td>
<td>M414T</td>
<td>resistance</td>
<td>0.4</td>
<td>Polymorphisms selected for SOF resistance</td>
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<td>Sofosbuvir (SOF)</td>
<td>F415Y</td>
<td>possible resistance</td>
<td>41.3</td>
<td>Polymorphism in the populations</td>
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<tr>
<td>Sofosbuvir (SOF)</td>
<td>I434L</td>
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<td>2.8</td>
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<tr>
<td>Sofosbuvir (SOF)</td>
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<td>2.8</td>
<td>G1a</td>
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<td>possible resistance</td>
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<td>Resistance ?</td>
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<td>Polymorphism in G1b</td>
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<td>Sofosbuvir (SOF)</td>
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<td>resistance ?</td>
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<td>Sofosbuvir (SOF)</td>
<td>A15G</td>
<td>possible resistance</td>
<td>2.40</td>
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</tbody>
</table>

N = 252; Torimiro et al, 2016
Conclusion

• Sofosbuvir resistant-associated HCV variants are not common in treatment-naive individuals in Cameroon.

• HCV genetic diversity is broad within this population.

Recommendation

• Surveillance of drug resistant HCV variants in DAA-naïve population to guide development of National Treatment Guidelines

LESSON LEARNT FROM THE AIDS PANDEMIC!
Study team

- Judith N. Torimiro
- Henry Luma
- Mathurin Kowo
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Thank you for your attention