

# High and Low Barriers to Resistance

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1. Mutations associated with nucleoside analogue NS5B inhibitors and Cyclophilin inhibitors are difficult to select.
2. Mutations associated with resistance to non-nucleoside NS5B inhibitors, protease inhibitors, and NS5A inhibitors are rapidly and frequently selected in vitro and in vivo.

# Definition of the “Genetic” Barrier: Number and Nature of Nucleotide Changes?

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Arg		Lys
A		A
G		A
G		G

R155K HCV NS5B 1a

Arg		Lys
C		A
G		A
G		G

R155K HCV NS5B 1b

# Fidelity of HCV NS5B

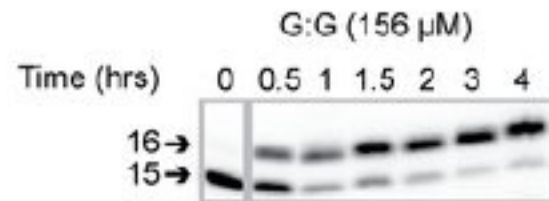
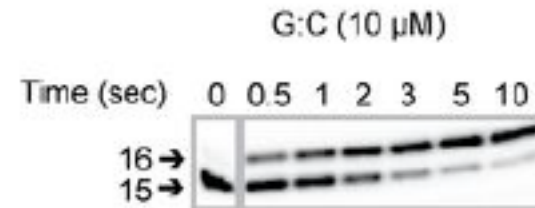
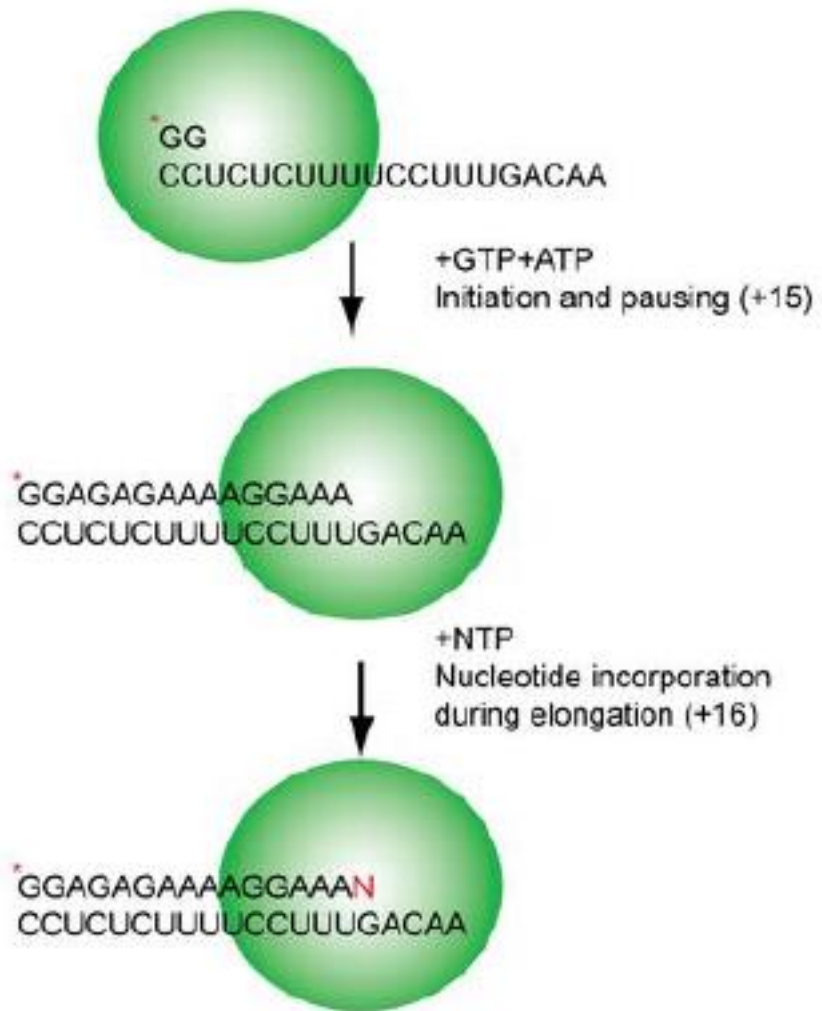
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How sloppy is the polymerase?

Are certain mutations preferred over others?

# Efficiency of Mismatch Formation

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# Error Rates

Template Base	NTP	$k_{pol}/K_d$ ( $\mu\text{M}^{-1}\text{sec}^{-1}$ )	Error rate /site	Error rate /genome
<b>A</b>	UTP	$5.6 \cdot 10^{-2}$	ref	
	ATP	$1.0 \cdot 10^{-6}$	$1.8 \cdot 10^{-5}$	0.034
	CTP	$5.1 \cdot 10^{-6}$	$9.2 \cdot 10^{-5}$	0.1
	GTP	$8.0 \cdot 10^{-8}$	$1.4 \cdot 10^{-6}$	0.003
<b>C</b>	GTP	$9.0 \cdot 10^{-2}$	ref	
	ATP	$2.1 \cdot 10^{-7}$	$2.3 \cdot 10^{-6}$	0.007
	CTP	$2.1 \cdot 10^{-6}$	$2.4 \cdot 10^{-5}$	0.068
	UTP	$6.2 \cdot 10^{-6}$	$6.9 \cdot 10^{-5}$	0.2
<b>G</b>	CTP	$1.4 \cdot 10^{-1}$	ref	
	ATP	$2.3 \cdot 10^{-6}$	$1.6 \cdot 10^{-5}$	0.044
	GTP	$3.1 \cdot 10^{-6}$	$2.1 \cdot 10^{-5}$	0.058
	UTP	$4.6 \cdot 10^{-4}$	$3.2 \cdot 10^{-3}$	9
<b>U</b>	ATP	$3.5 \cdot 10^{-2}$	ref	
	CTP	$5.8 \cdot 10^{-7}$	$1.7 \cdot 10^{-5}$	0.034
	GTP	$3.0 \cdot 10^{-4}$	$8.7 \cdot 10^{-3}$	18
	UTP	$5.8 \cdot 10^{-7}$	$1.7 \cdot 10^{-5}$	0.035

Error rate per site =  $(k_{pol}/K_d)_i / ((k_{pol}/K_d)_i + (k_{pol}/K_d)_c)$

Error rate per genome = error rate per site x frequency of corresponding base in genome

# HCV Evolution in Cell-based Assays

(Kato et al. 2005)

		Base substitution	% total substitutions (n=448)	Possible Pathways			
Transitions	A-G		26	A-G	A:C:G	or	<u>A:U:G</u>
	U-C		24	U-C	<u>U:G:C</u>	or	U:A:C
	C-U		13	C-U	C:A:U	or	<u>C:G:U</u>
	G-A		9	G-A	<u>G:U:A</u>	or	G:C:A
Transversions	A-C		7				
	U-G		6				
	G-C		5				
	A-U		3				
	C-A		3				
	C-G		2				
	U-A		1				
	G-U		1				

# 454 Deep Sequencing Analysis

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20 HCV (1a/1b) infected, treatment naïve patients

HCV 1a replicon control

Amplicons derived from NS3

Deep sequencing was performed at Virco DBA (Virco, Belgium)

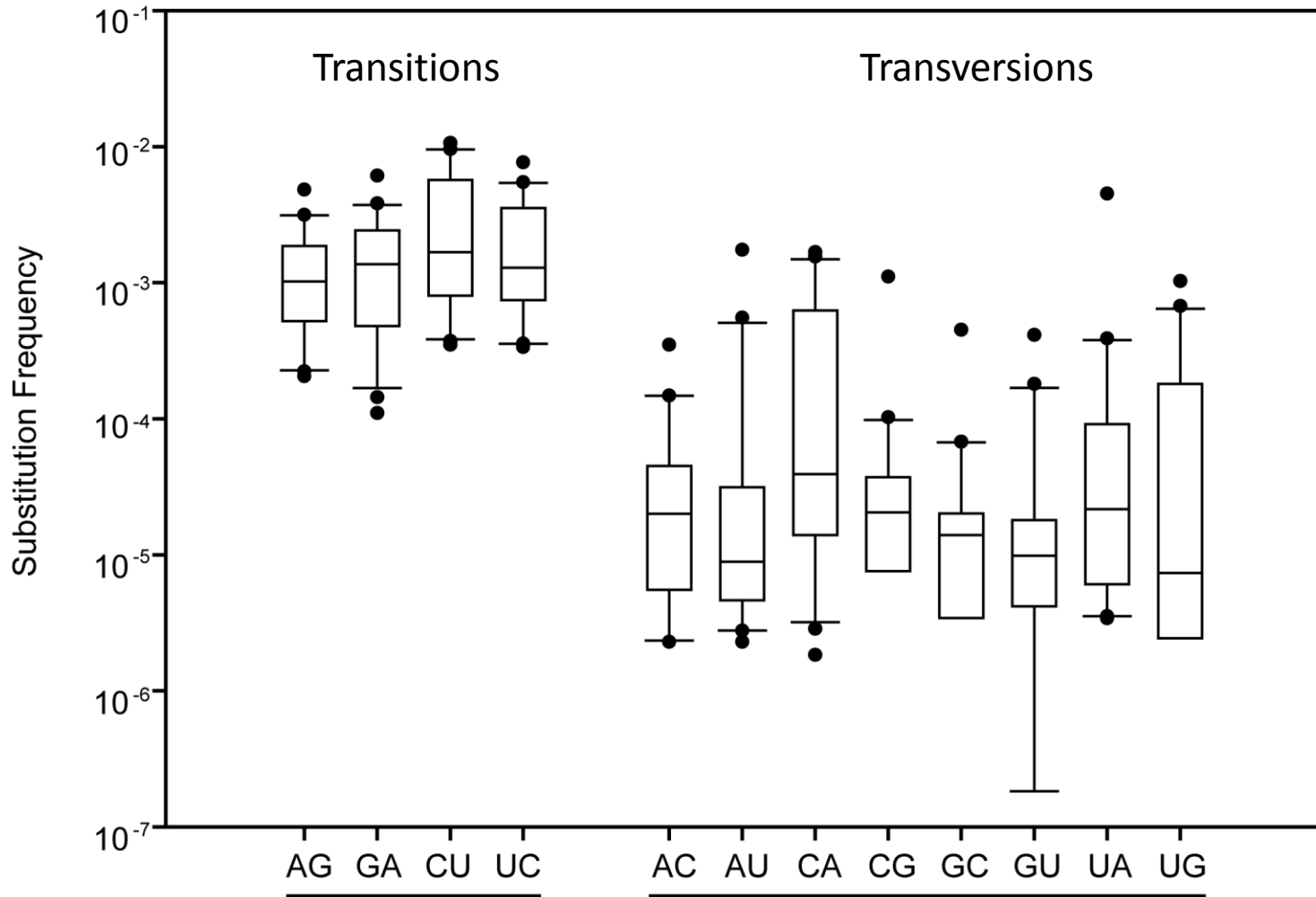
Average number of base-read-positions per patient: 781,844

Average base changes per patient: 7,385 (~150,000 total)

Plasmid (PCR) control: 3,764,286 base-read-positions, 67 mutations

# Frequencies of Transitions and Transversions

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# High Genetic Barriers

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## Nucleoside Analogue NS5B and Cyclophilin Inhibitors

Ser		Thr
(+)	(-)	(+)
A	U	A
G	G	C
C	G	C

Ser		Thr
(+)	(-)	(+)
A	U	A
G	C	C
C	G	C

S282T NS5B

Asp		Glu
(+)	(-)	(+)
G	C	G
A	U	A
U	U	A

Asp		Glu
(+)	(-)	(+)
G	C	G
A	U	A
U	A	A

D320E NS5A

# Low Genetic Barriers

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Often associated with:

Non-Nucleoside Analogues, Protease Inhibitors, NS5A Inhibitors

Cys		Tyr
(+)	(-)	(+)
U	A	U
G	U	A
C	G	C

Cys		Tyr
(+)	(-)	(+)
U	A	U
G	C	A
C	G	C

C316Y NS5B

# “Mixed” Genetic Barriers

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Asp		Gly
(+)	(-)	(+)
G	C	G
A	C	G
C	G	C

**D168G NS3**  
“Early  
selection at  
low dose”

Asp		Gly
(+)	(-)	(+)
G	C	G
A	U	G
C	G	C

Asp		Ala
(+)	(-)	(+)
G	C	G
A	G	C
C	G	C

**D168A NS3**  
“Late selection  
at high dose”

Asp		Ala
(+)	(-)	(+)
G	C	G
A	U	C
C	G	C

# Conclusions

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- The biochemical data correlate with deep sequencing analyses of HCV RNA samples from treatment naïve patients that much higher frequencies of transitions over transversions.
- The nature— and NOT ONLY the number of nucleotide changes - may therefore contribute to the genetic barrier development of drug resistance.
- The high barrier to the selection of S282T (or D320E) may be ascribed to combined effects of fitness deficits, limited benefits conferred through low-levels of resistance and/or the high genetic barrier associated transversions.
- The genetic barrier could be exploited in choosing potent drug combinations.

# McGill University

## Department of Microbiology and Immunology

- EgorTchesnokov
- Megan Powdrill
- Greg Beilhartz
- Suzanne McCormick
- Mia Biondi
- Jean Bernatchez
- Chris Ablenas
- SveaRawe
- Robert Kozak
- Nicholas Bennet
- AnupriyaKulkarni
- Jenaya Rickard
- Anick Auger

### Princeton University

Roger Kouyos



### Gilead Sciences

Evguenia S. Svarovskaia  
Ross Martin  
Hongmei Mo



National Canadian  
Research Training Program in  
Hepatitis C



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sur le cancer  
The Cancer Research  
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