

Supplemental Digital Content 1: Primers Used in Site-Directed Mutagenesis Studies for

Amplification of Specific Mutations

Mutation	Primer
K103N	Fw 5'-CCTGCAGGGTAAACACAGA <u>ACA</u> AATCAGTAACAGTACTGGA-3' Rev 5'-TCCAGTACTGTTACTGATT <u>GTT</u> CTGTTAACCCCTGCAGG-3'
Y181C	Fw 5'-CAAAATCCAGACATAGTCATC <u>TGT</u> CAATACATGGATGATTG-3' Rev 5'-CAAATCATCCATGTATT <u>GACAGA</u> TGACTATGTCTGGATTTG-3'
G190A	Fw 5'-CATGGATGATTGTATGT <u>AGC</u> ATCTGACTTAGAAATAGGGC-3' Rev 5'-GCCCTATTCTAAC <u>GT</u> CAGAT <u>TG</u> CTACATA <u>ACAA</u> ATCATCCATG-3'
E138K	Fw 5'-GCATTCACTATA <u>CC</u> TTAGTGAA <u>ACA</u> AT <u>AAG</u> ACACCAGGGATT-3' Rev 5'-AATCCCTGGTGT <u>CTT</u> ATTGTTACACTAGGTATA <u>GT</u> GAATGC-3'
E138A	Fw 5'-CCATACCTAGTATA <u>AA</u> ACAAT <u>GCG</u> ACACCAGGGATTAGATATC-3' Rev 5'-GATATCTAAC <u>CC</u> CTGGTGT <u>CG</u> CATTGTTATA <u>CT</u> AGGTATGG-3'
E138V	Fw 5'-TAGTATA <u>AA</u> ACAAT <u>GTG</u> ACACCAGGGATTAGATAT-3' Rev 5'-ATATCTAAC <u>CC</u> CTGGTGT <u>CAC</u> ATTGTTATA <u>CT</u> ACTA-3'
M184I	Fw 5'-CATCTATCA <u>AA</u> AC <u>ATT</u> GATGATTGTA-3' Rev 5'-TACAAATCAT <u>CA</u> AT <u>GT</u> TATTGATAGATG-3
M184V	Fw 5'-CATCTATCA <u>AA</u> AC <u>GTG</u> GATGATTGTA-3' Rev 5'-TACAAATCAT <u>CC</u> <u>AC</u> GTATTGATAGATG-3'

Supplemental Digital Content 2: Mutations Selected by EFV and EFV/TFV Drug Pressure After 20-30 Weeks.			
Virus	Baseline Genotype (Subtype)	Week 20-30	
		Drug (nM)	Acquired Mutation s
NL-4.3 ^a	wt (B)	EFV (1000)	K101E, V108I, V189I/V
NL-4.3 ^b	Wt (B)	EFV (200)	K103N, V189I/V, E399G
NL-4.3	K103N (B)	EFV (500)	<u>K103N</u> , Y318F, N348I/N
NL-4.3 ^a	Y181C (B)	EFV (20)	V75I, K101Q, V108I, <u>Y181C</u>
NL-4.3 ^b	Y181C (B)	EFV (100)	V75L, V106I, <u>Y181C</u> , Y188H, A400A/T
NL-4.3	K103N/Y181C (B)	EFV (50)	<u>K103N</u> , <u>Y181C</u>
NL-4.3	K103N/G190A (B)	EFV (10)	<u>K103N</u> , <u>G190A</u>
NL-4.3	G190A (B)	EFV (7.5)	A62V, V106A, V179D/V, <u>G190A</u>
NL-4.3	E138K (B)	EFV (90)	L100I, K103K/N, <u>E138K</u>
NL-4.3	M184I(B)	EFV (60)	L100I/L, <u>M184I</u> , H188H/Y
NL-4.3	E138K/M184I (B)	EFV (90)	<u>E138K</u> , G190A
NL-4.3	E138K/M184V (B)	EFV (40)	<u>E138K</u> , E399G
NL-4.3	M184V (B)	EFV (50)	Y188C/Y
4743	G190A (C)	EFV (1000)	V106M, <u>G190A</u> , F227F/L, E399K
10680	wt (C)	EFV (10000)	V106M, Y188C, K275R
10680	Y181C (C)	EFV (10000)	A62A/V, K101E/K, V106M, <u>Y181C</u> , F227L, L283L/V
10680	wt (C)	EFV (250) TFV (500)	V106M, F227L
10680	Y181C (C)	EFV (1000) TFV (1000)	V106M, <u>Y181C</u>

Genotypic analysis was done at weeks 20, 25 or 30. Baseline mutations that persisted at time of genotypic analysis are underlined. Amino acid substitutions at position 184 in all clones containing M184V, E138K/M184V and E138K/M184I reverted to wt, while amino acid substitutions in the M184I clone were retained in the presence of EFV pressure.

Supplemental Digital Content 3: Mutations Selected by Serial Passage Experiments in CBMCs in the Presence of TMC120 Drug Pressure			
Virus	Baseline Genotype	Week 20	
		DPV Conc (nM)	Acquired Mutations
NL-4.3	wt	100	E138K, L100I
NL-4.3	E138K	70	<u>L100I</u> , <u>E138K</u>
NL-4.3	E138K/M184I	200	<u>L100I</u> , <u>E138K</u> , <u>M184I</u>
NL-4.3	M184I	80	<u>E138K</u>
NL-4.3	E138K/M184V	90	V108I, <u>E138K</u>
NL-4.3	M184V	70	E138K

Baseline mutations that persisted at time of genotypic analysis are underlined. Amino acid substitutions at position 184 in the clones M184V, M184I and E138K/M184V reverted to wt, while amino acid substitutions in the E138KM184I clone were retained in the presence of TMC120.