CONFERENCE CAHR 2020

CONGRÈS DE L'ACRV 2020

A clinically-derived HIV Integrase Sequence Harboring High Levels of Genotypic and Phenotypic Resistance to Integrase Strand Transfer Inhibitors

Peter K. Cheung[1, 2], Aniqa Shahid[1, 2], Winnie Dong[1], Katherine J. Lepik[1, 3], Mark A. Brockman[1, 2], Zabrina L. Brumme[1, 2], Chanson J. Brumme[1, 4]

1. BC Centre for Excellence in HIV/AIDS, Vancouver, BC, Canada, 2. Faculty of Health Sciences, Simon Fraser University, Burnaby, BC, Canada, 3. Pharmacy Department, St. Paul's Hospital, Vancouver, BC, Canada, 4. Division of Infectious Diseases, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada

Program ID#: CS3.06

Track: Clinical Sciences

contact: pcheung@cfenet.ubc.ca

I HAVE NO CONFLICTS OF INTEREST

BRITISH COLUMBIA CENTRE *for* EXCELLENCE *in* HIV/AIDS

SFU SIMON FRASER UNIVERSITY

Providence HEALTH CARE How you want to be treated. BRITISH COLUMBIA Ministry of Health

Phenotypic Analysis of a Unique Clinically-derived Sample Harboring High Levels of Integrase Strand Transfer Inhibitor Resistance

Routine clinical INSTI genotyping identified a sample harboring T97A, E138K, G140S and Q148H which conferred high-level INSTI resistance

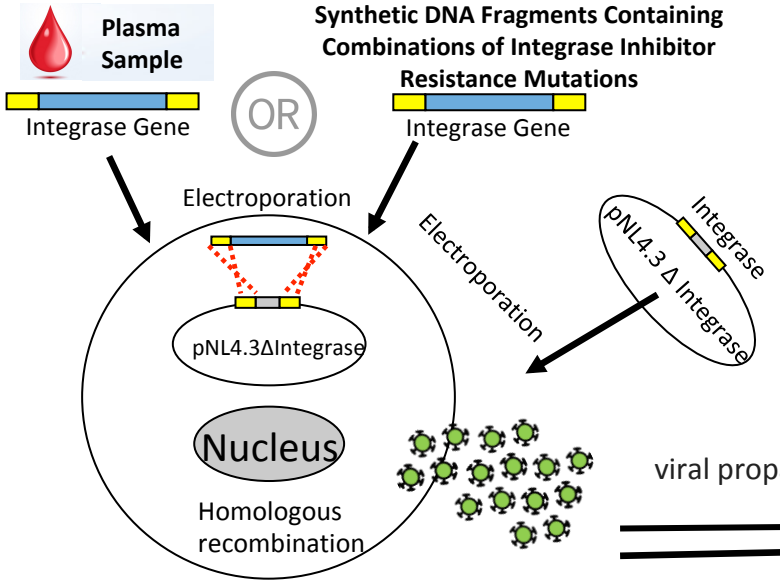
Drug resistance interpretation: IN **(Stanford University HIVdb Program Report)**

IN Major Resistance Mutations:	**E138K, G140S, Q148H**
IN Accessory Resistance Mutations:	**T97A**
Other Mutations:	S24N, I60M, L68I, I72V, S119P, T122I, T124N, M154I, T206S, D288S

Integrase Strand Transfer Inhibitors	
bictegravir (BIC)	High-Level Resistance
dolutegravir (DTG)	High-Level Resistance
elvitegravir (EVG)	High-Level Resistance
raltegravir (RAL)	High-Level Resistance

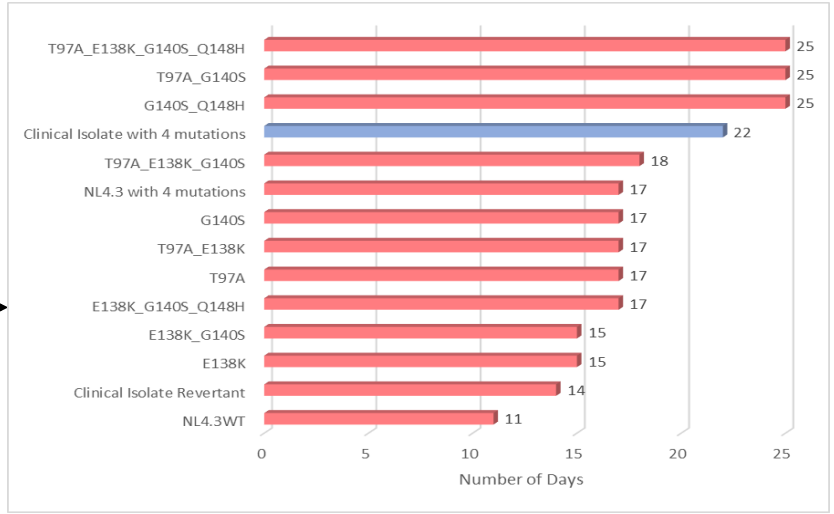
Subsequent single-genome amplification of integrase from the original plasma sample yielded three identical sequences, all harboring this mutation combination.

Chimeric Virus Construction and Resistance Phenotyping



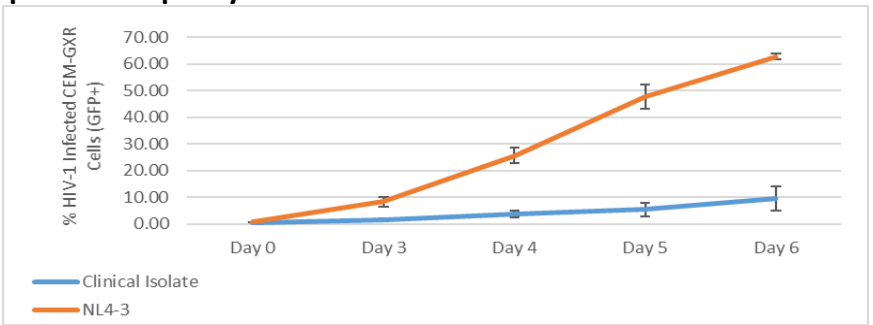
Chimeric Viruses are constructed by co-transfection of the patient-derived integrase amplicon **or** one of 17 synthetic DNA fragments containing all possible combinations of 0-, 1-, 2-, 3-, or 4-mutation combinations in the clinical sample background, with a linearized integrase-deleted NL4.3 plasmid, into a *tat*-driven GFP reporter CEM-GXR cell line. Viruses are harvested when GFP+ (HIV-infected) cells reach >15% in culture. Viruses are titered and then assessed for replication and drug resistance in the same cell line.

Time required to generate recombinant viruses is an estimate of viral replication capacity



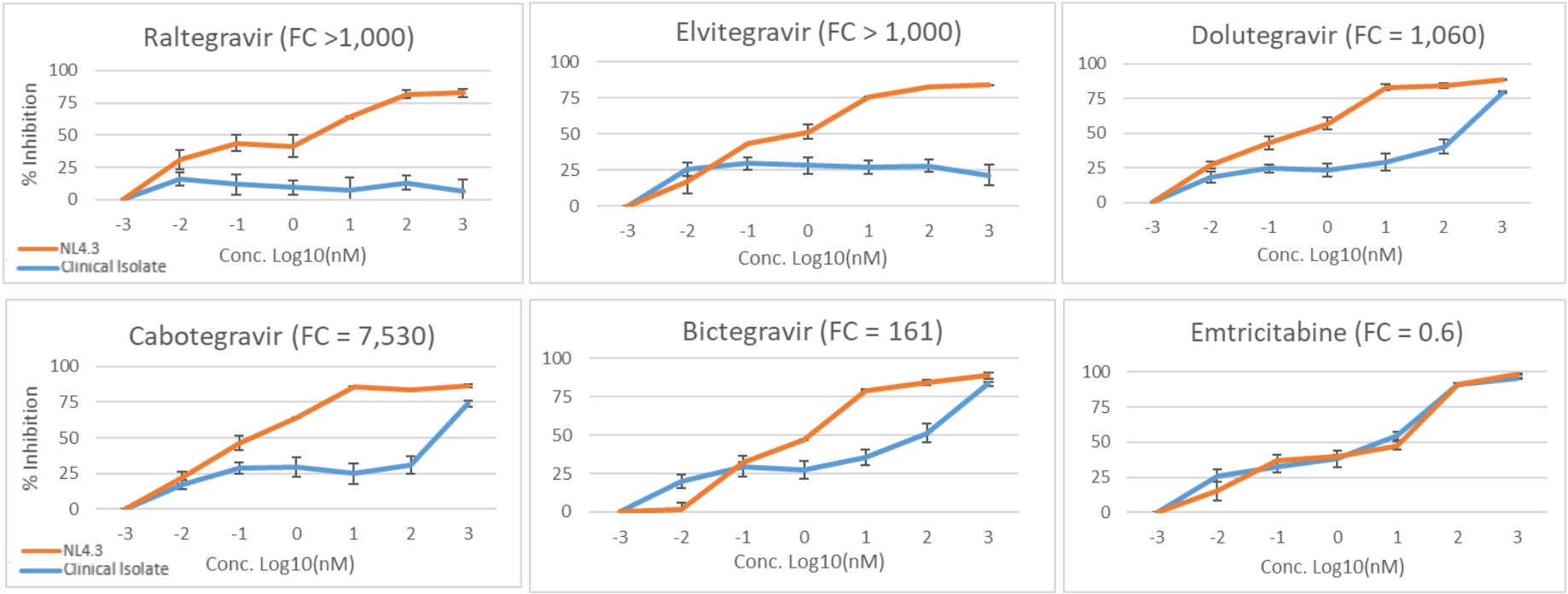
The following mutation combinations did not produce replication-competent HIV, suggesting they are not viable or incompatible with the NL4.3 backbone: Q148H; T97A Q148H; E138K Q148H; T97A E138K Q148H; T97A G140S Q148H

Replication capacity of the clinical isolate and NL43 reference strain



Replication capacity of the chimeric virus derived from the clinical isolate was significantly impaired compared to NL4.3, confirming that time required to generate recombinant viruses is a surrogate of replication capacity

Substantial Decreases in Phenotypic Susceptibility to Integrase Inhibitors of a Chimeric Virus Expressing Clinically-Derived Integrase



FC = Fold Change in EC_{50} relative to NL4.3 reference virus

Prior to phenotypic assessment, the entire HIV genome of the 3 recombinant viruses encoding the identical clonal integrase sequence from the clinical isolate were single-genome amplified and deep-sequenced by Illumina MiSeq. These were re-confirmed to be clonal over the integrase region, and no reproducible mutations occurred elsewhere in the HIV genome. Phenotyping assays were performed in triplicate.

Figures show % inhibition of recombinant virus replication in the presence of increasing concentrations of Integrase inhibitors raltegravir, elvitegravir, dolutegravir and bictegravir relative, to a NL4.3 reference virus. % inhibition of emtricitabine, a NRTI, was used as a negative control.

The clinically-derived integrase recombinant virus, which harbored T97A, E138K, G140S, Q148H, exhibited essentially complete resistance to raltegravir and elvitegravir, and 1060-, 7530- and 161-fold decreased susceptibility to dolutegravir, cabotegravir and bictegravir, respectively. No change in emtricitabine susceptibility was observed.

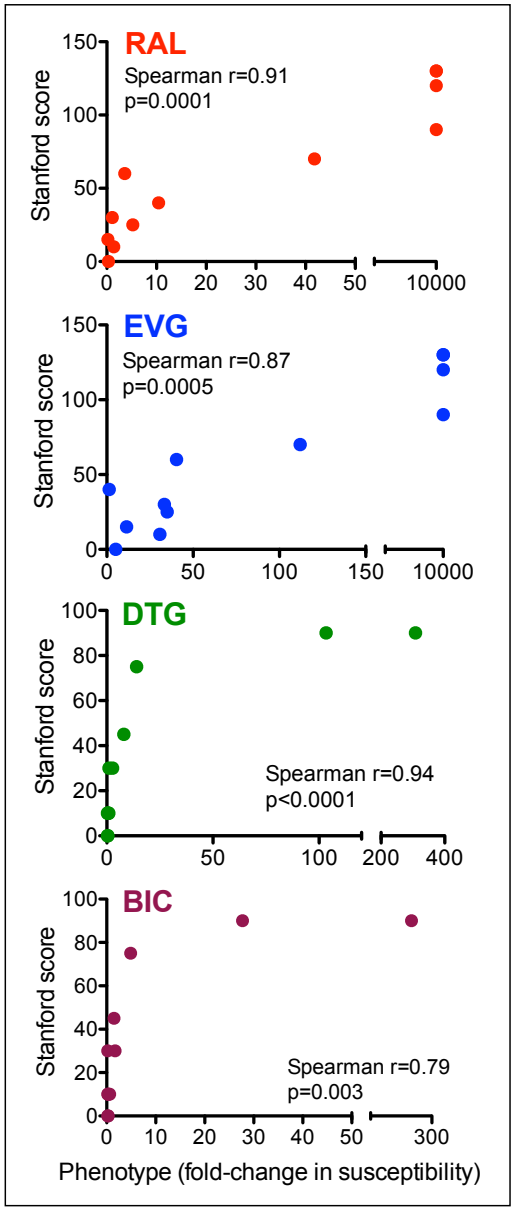
Substantial Variation in Phenotypic Susceptibility to INSTI of Chimeric Viruses Containing INT Mutation Combinations

Integrase Mutations	RAL FC	EVG FC	DTG FC	CAB FC	BIC FC	FTC FC
T97A E138K G140S Q148H	>10,000	>10,000	308	2730	250	1.1
E138K G140S Q148H	>10,000	>10,000	14	177	4.9	1.2
G140S Q148H	>10,000	>10,000	8.0	122	1.5	0.5
Clinical Isolate Revertant	0.3	5.1	0.5	0.6	0.3	1.0
E138K	0.2	11.4	0.3	0.7	0.3	0.6
T97A	1.4	30.7	0.3	0.4	0.2	0.6
G140S	1.1	33.3	0.7	0.7	0.6	0.8
E138K G140S	3.6	40.4	1	1.5	0.2	0.8
T97A E138K	5.2	34.9	0.5	0.8	0.4	1.2
T97A G140S	10.4	19.4	0.9	5.4	0.2	0.7
T97A E138K G140S	41.8	112	2.7	6.3	1.7	1.2
NL4.3 with T97A E138K G140S Q148H	>10,000	>10,000	103	898	27.7	1.1
NL4.3WT	1.0	1.0	1.0	1.0	1.0	1.0

Table Above: Fold Change (FC) in RAL, EVG, DTG, CAB, BIC, and FTC EC_{50} relative to NL4.3 Wild-type of recombinant viruses harboring different combinations of T97A, E138K, G140S, and Q148H in the background of the clinical isolate.

Viruses containing Q148H plus one or more additional mutations eliminated RAL and EVG activity and conferred high-level resistance to DTG, CAB and BIC.

Figure at right: Observed RAL, EVG, DTG and BIG EC_{50} Fold Change correlated significantly with Stanford University HIVdb Resistance Scores (algorithm v8.9-1). HIVdb classifies estimated levels of resistance as follows: **Susceptible**: Total score 0 to 9; **Potential low-level resistance**: Total score 10 to 14; **Low-level resistance**: Total score 15 to 29; **Intermediate resistance**: Total score 30 to 59; **High-level resistance**: Total score >= 60.



Conclusions

- The present study represents the first phenotypic characterization of the impact of T97A/E138K/G140S/Q148H, and combinations thereof, on INSTI phenotypic resistance.
- Chimeric virus containing a clinically-derived integrase sequence harboring T97A/E138K/G140S/Q148H mutations in an NL4.3 backbone was essentially completely resistant to RAL and EVG, and exhibited extremely high-level phenotypic resistance to DTG, CAB, and BIC.
- This chimeric virus however exhibited substantially impaired replication capacity in the absence of drug.
- Engineering these 4 mutations into NL4.3 also produced a virus that was essentially completely resistant to RAL and EVG, and exhibited high-level phenotypic resistance to DTG, CAB, and BIC
- Chimeric viruses containing single Integrase mutations T97A, E138K, or G140S did not have reduced susceptibility to DTC, CAB or BIC.
- Many chimeric viruses containing the Q148H, alone or in combination, did not produce viable viruses, suggesting severely impaired fitness in the absence of compensatory mutations
- The dual mutation G140S/Q148H was sufficient to eliminate RAL and EVG activity, and conferred 8.0-, 122-, and 1.5-fold decreased susceptibility to DTG, CAB, and BIC respectively
- The triple mutation G140S/Q148H/E138K eliminated RAL and EVG activity further reduced susceptibility to DTG, CAB, and BIC was further reduced when compared to G140S/Q148H alone.