CONFERENCE CAHR 2021

CONGRÈS DE L'ACRV 2021

Evaluation of Combinations of Clinical Integrase Mutations on Integrase Strand Transfer Inhibitor Resistance

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#: 166

Track: Clinical Sciences

contact: pcheung@bccfe.ca

I HAVE NO CONFLICTS OF INTEREST

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

Providence HEALTH CARE How you want to be treated.

BRITISH COLUMBIA Ministry of Health

Background

- Although resistance mutations have been identified *in vitro* and in patients failing integrase strand transfer inhibitor (INSTI) treatment, less is known about the effect of ***combinations*** of these mutations on INSTI phenotypic resistance

Objective

- To measure the combinatorial effects of major Integrase resistance mutations on INSTI phenotypic resistance

Approach

- Starting with a clinical isolate harboring multiple resistance mutations conferring high genotypic resistance to all INSTIs, we constructed chimeric viruses harboring all possible combinations of these mutations to quantify their individual and combined effects on INSTI phenotypic susceptibility

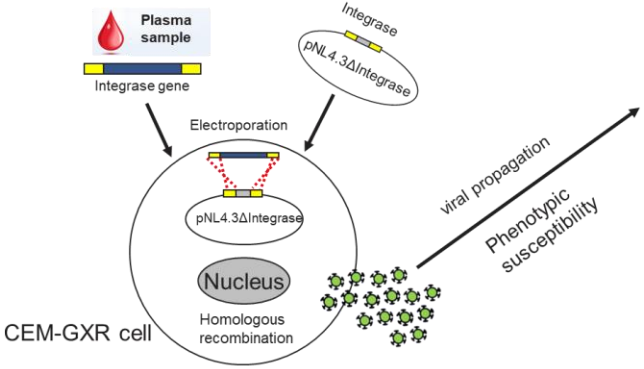
Clinical isolate, recombinant virus construction and resistance phenotyping

1. Clinical Isolate: Routine clinical INSTI genotyping identified a sample harboring T97A, E138K, G140S and Q148H which together confer high-level INSTI resistance

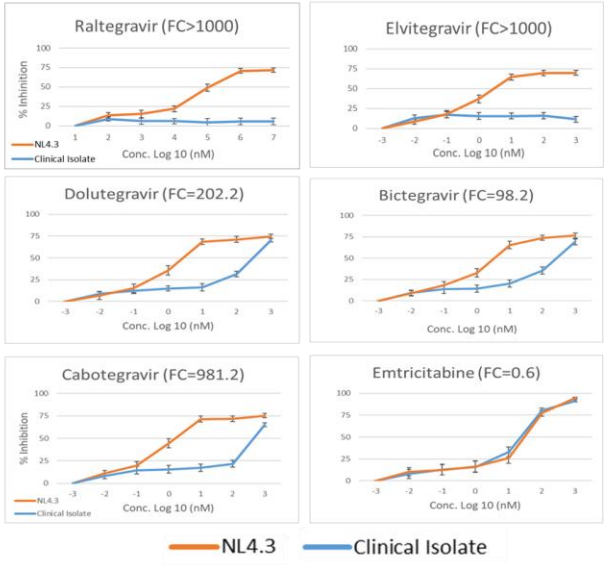
1. Clinical isolate

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

2. Recombinant virus construction



3. Resistance phenotyping



2. Recombinant Virus Construction: Chimeric Viruses are constructed by co-transfection of the patient-derived integrase amplicon **or** one of the synthetic DNA fragments containing all possible combinations of 0-, 1-, 2-, 3-, or 4-mutation combination 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 assessed for drug resistance in the same cell line.

3. Resistance Phenotyping: The above Figures show the INSTI resistance phenotypes data of the clinical isolate. The orange line represents the NL4.3 wild-type reference virus and the blue line represents the clinical isolate carrying the four mutations. Both RAL and EVG in the top two figures showed no activity against this clinical isolate. For DTG, CAB and BIC, the blue line is shifted to the right indicating a decrease in susceptibility to these inhibitors. % inhibition of emtricitabine, a NRTI, was used as a negative control. FC = Fold Change in EC_{50} relative to NL4.3 reference virus.

Construction and phenotypic assessment of recombinant virus panel

1. Mutant virus panel harboring all mutation combinations:
The 4 substitutions were deconstructed into all possible combinations with either the autologous backbone or a NL4.3 backbone using commercially available DNA synthesis. The resulting viruses were verified by Illumina DNA sequencing to ensure the absence of in vitro mutations. The isolate revertant variant was generated by reversing these 4 substitutions back to the subtype B consensus residues.

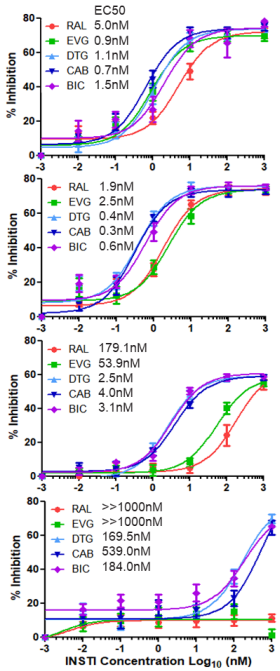
1. Mutant virus panel harboring all mutation combinations

Virus	Backbone	Mutations	Outcome
1	Autologous	E138K	✓
2	Autologous	T97A	✓
3	Autologous	G140S	✓
4	Autologous	Q148H	✗
5	Autologous	E138K_G140S	✓
6	Autologous	E138K_Q148H	✗
7	Autologous	T97A_E138K	✓
8	Autologous	T97A_G140S	✓
9	Autologous	T97A_Q148H	✗
10	Autologous	G140S_Q148H	✓
11	Autologous	E138K_G140S_Q148H	✓
12	Autologous	T97A_E138K_G140S	✓
13	Autologous	T97A_E138K_Q148H	✗
14	Autologous	T97A_G140S_Q148H	✓
15	Autologous	T97A_E138K_G140S_Q148H	✓
16	HIV-$1_{NL4.3}$	T97A_E138K_G140S_Q148H	✓
17	Autologous	Isolate revertant	✓
18	HIV-$1_{NL4.3}$	NL4.3 WT	✓

✓ Virus generated and sequence-validated ✗ virus failed to grow, or grew only after *in vitro* mutation

Consistent with the known fitness impact of Q148H and its compensation by G140S, viruses engineered with Q148H without G140S failed to propagate or did so only after acquiring additional mutations *in vitro*.

2. Phenotypic INSTI resistance: representative data



NL4.3 WT

Susceptible to all INSTI

Clinical Isolate Revertant

Susceptible to
RAL, DTG, CAB, BIC
Low-level Resistance to EVG

T97A_E138_G140S

Low-level Resistance to
DTG, CAB, BIC
High-level Resistance to
RAL, EVG

T97A_E138K_G140S_ Q148H

High-level Resistance
to all INSTI

2. Phenotypic INSTI resistance:
Representative data from samples with a wide range of phenotypic resistance profiles.

As expected, NL4.3 is fully susceptible to all INSTIs.

The clinical revertant virus was also susceptible to all INSTIs except EVG which showed low-level resistance.

Chimeric virus that harboured the T97A/E138K/G140S combination exhibited low-level resistance to DTG, CAB, and BIC but high-level resistance to RAL, and EVG.

The chimeric virus harbouring the quadruple mutation combination conferred high-level resistance to all INSTIs .

EC50 fold-change values and agreement with predicted genotypic resistance scores

Table on the Left: Fold Change (FC) in RAL, EVG, DTG, CAB, and BIC 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 or NL4.3.

	RAL		EVG		DTG	CAB		BIC
Mutation Profile	**EC50 FC (95%CI)**		**EC50 FC (95%CI)**		**EC50 FC (95%CI)**	**EC50 FC (95%CI)**		**EC50 FC (95%CI)**
E138K	0.4 (0.2-0.6)	**	2.4 (1.1-5.3)		0.2 (0.1-0.2)	0.3 (0.15-0.8)		0.2 (0.1-0.4)
T97A	1.6 (0.7-3.4)	**	14.0 (6.9-28.4)		0.1 (0.1-0.2)	0.2 (0.15-0.3)		0.1 (0.1-0.2)
G140S	1.3 (0.7-2.7)		15.4 (5.3-44.7)		0.3 (0.1-0.6)	0.5 (0.3-0.8)		0.3 (0.1-0.6)
E138K_G140S	3.2 (1.8-5.5)	**	16.9 (8.1-35.2)		0.4 (0.3-0.7)	0.8 (0.4-1.8)	**	0.3 (0.2-0.4)
T97A_G140S	13.1 (7.1-24.1)	**	21.8 (11.6-40.8)		0.9 (0.6-1.3)	3.5 (1.7-7.2)	**	0.8 (0.5-1.3)
T97A_E138K	4.5 (1.7-11.6)		20.1 (11.0-36.5)		0.3 (0.2-0.5)	0.5 (0.3-1.0)		0.2 (0.1-0.4)
G140S_Q148H#	>1000		>1000		8.3 (4.6-15.1)	50.9 (24.3-106.8)		3.1 (1.9-5.1)
T97A_E138K_G140S#	35.5 (17.5-72.4)		58.8 (36.6-94.2)		2.2 (1.0-3.2)	6.1 (4.0-9.3)		2.0 (1.4-2.9)
E138K_G140S_Q148H	>1000		>1000	**	12.1 (5.7-25.4)	98.6 (40.8-238.2)	**	4.6 (1.9-11.0)
T97A_G140S_Q148H	>1000		>1000	**	107.9 (52.5-221.7)	498.6 (139.2-1786.0)	**	47.7 (16.1-140.6)
T97A_E138K_G140S_Q148H	>1000		>1000		153.8 (77.2-306.7)	817.9 (162.4-4118.4)	**	120.1 (45.5-317.0)
NL4.3-T97A_E138K_G140S_Q148H	>1000		>1000		66.2 (23.2-188.8)	268.6 (99.8-723.4)	**	8.2 (3.5-19.3)
Clinical Revertant	0.4 (0.2-0.6)		2.8 (1.5-5.1)		0.3 (0.2-0.6)	0.5 (0.3-0.7)		0.4 (0.2-0.7)
NL4.3WT	1.0		1.0		1.0	1.0		1.0

** | p<0.05 in a pairwise comparison

Take-home findings:

- In general, higher numbers of mutations is associated with increasing phenotypic resistance, with the exceptions noted by the # symbol.
- When present in single, double, and triple mutation combinations, T97A conferred higher resistance than E138K as noted by the ** symbols.
- The quadruple mutation in the autologous backbone conferred higher resistance in DTG, CAB and BIC when compared to the quadruple mutation in NL4.3 backbone as noted by the ** symbols, suggesting that other polymorphisms in the autologous backbone further increased resistance.
- The measured EC50 fold-change values for all INSTIs correlated strongly with Stanford HIVdb resistance scores.

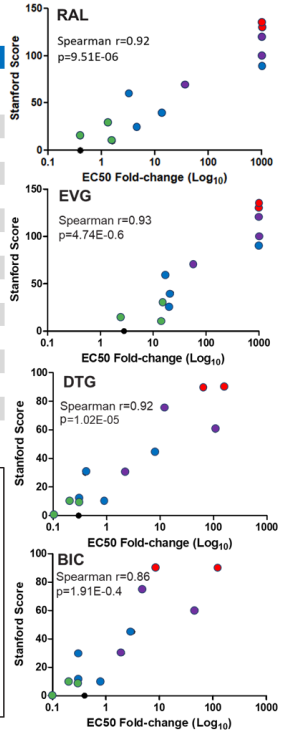


Figure on the right: Observed RAL, EVG, DTG and BIG EC_{50} Fold Change correlated significantly with Stanford University HIVdb Resistance Scores (algorithm v8.9-1).

Conclusions

- Panels of chimeric viruses can be used to assess the cumulative effect of combinations of Integrase mutations on INSTI susceptibility

- We confirm that in this clinical isolate, Q148H plus one of T97A or E138K is required in order to confer high-level resistance to DTG, CAB and BIC

- G140S must be present to compensate for a fitness defect conferred by Q148H