Determination of Integrase Inhibitor Resistance using a Novel HIV Phenotype Assay

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Background

- Integrase strand transfer inhibitors (INSTI) such as raltegravir (RAL), elvitegravir (EVG) and dolutegravir (DTG) are becoming more common in treatment regimens highlighting the need for a robust database for INSTI resistance prediction based on genotype.
- Matching genotype and phenotype data from diverse integrase resistant HIV is required for this database.
- A large number of diverse INSTI resistant viruses are necessary to cover relevant mutations in (1).
- Commercial INSTI phenotype assays are available but are hindered by high expense.

MUTATIONS IN THE INTEGRASE GENE ASSOCIATED WITH RESISTANCE TO INTEGRASE STRAND TRANSFER INHIBITORS

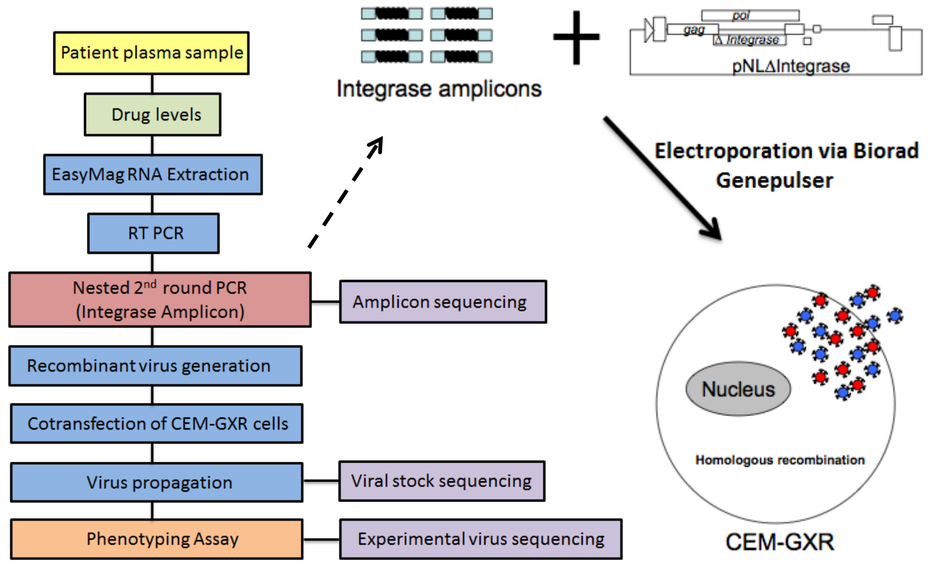
Dolutegravir[aa]						E 138 A K	G 140 S A		Q 148 H			
Elvitegravir[bb]	T 66 I A K		E 92 Q G	T 97 A				S 147 G	Q 148 R H K	N 155 H		
Raltegravir[cc]		L 74 M	E 92 Q	T 97 A	E 138 A K	G 140 A S	Y 143 R H C		Q 148 H K R	N 155 H		

Objectives

- Develop an in-house HIV INSTI phenotype assay
- Use oligogenome amplification to isolate diverse virus variants from patient samples
- Produce matched phenotypes with genotypes linked to clinical outcomes to create a predictive INSTI resistance database for resistance reports for physicians
- Better understand integrase resistance

Methods

- Retrospective plasma samples from 2 patients failing INSTI therapy (and drug present in sample) in Canada for routine BCCfE HIV drug resistance testing were phenotyped upon physician request.
- Recombinant viruses were made by cotransfection of patient derived integrase amplicons with linearized pNL4-3*Δintegrase* plasmid into a reporter T-cell line (CEM-GXR) that produces green fluorescent protein (GFP) when infected with HIV as described in (2).
- Percent GFP (%GFP) was monitored by flow cytometry as in (2).
- Virus supernatant was harvested when %GFP was 25-80%.
- Phenotype assays were performed when %GFP infected cells was >10%.



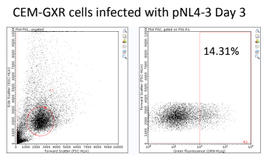
Phenotype Assay

- Added 1% virus infected cells to uninfected CEM-GXR cells (400,000 cells/ml) in 96 well plates on day 0 followed immediately by addition of INSTI drugs RAL, EVG and DTG 0.01 to 10,000 nM.
- Incubated assay 3-6 days and then %GFP determined using the Guava EasyCyte flow cytometer.

Plate Reading

- %GFP determined by counting fluorescent cells
- Cell with green fluorescence >100 units are labeled as infected

CEM-GXR cells infected with pNL4-3 Day 3



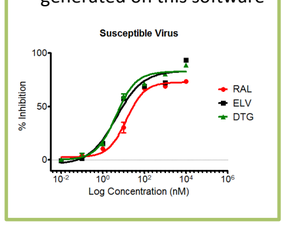
Data Output

- Based on GFP fluorescence of the healthy cells, Guava will generate a percent infected value for each well
- Percent inhibition values are calculated using %GFP values

$$\text{Percent Inhibition} = \left[1 - \frac{\text{Well with integrase inhibitor}}{\text{Average of wells with no drug}}\right] \times 100$$

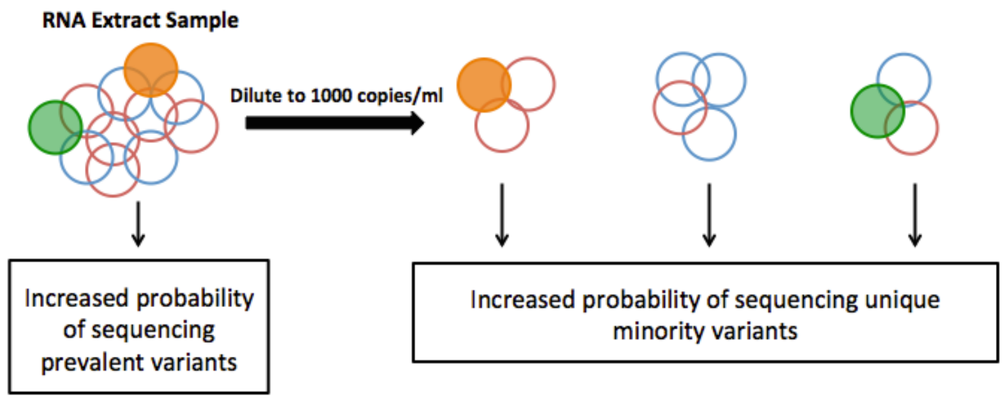
Data Analysis

- IC50 values and standard error are calculated using GraphPad Prism 5 software
- IC50 curves are also generated on this software



Oligogenome amplification

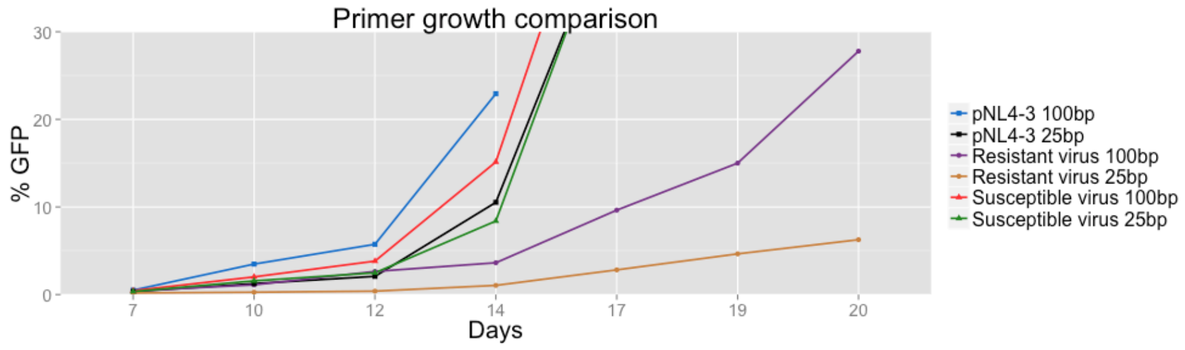
- Extracts of samples with demonstrated INSTI resistance (i.e. significant shifts in IC50) were diluted to a viral load equivalent to 1000 HIV RNA copies/mL prior to RT-PCR in replicates of 94.
- Successful integrase amplicons were sequenced by Sanger method on an ABI 3730.
- Unique INSTI amplicon variants will be phenotyped.
- Efficiency of amplification at this viral load was approximately ~ 80%



Results

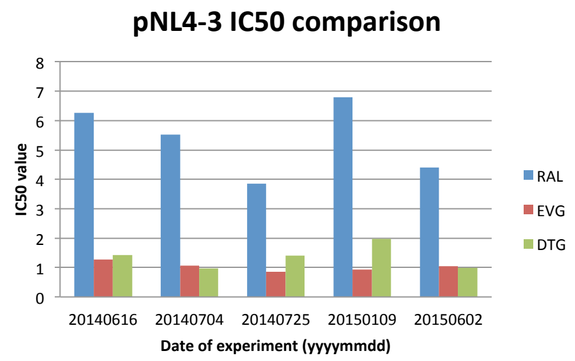
Effect of homology of amplicon to plasmid vector on virus growth rate

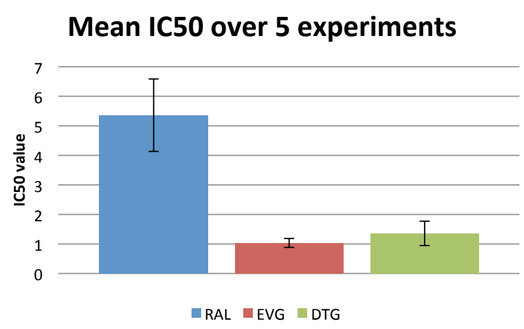
- NL4-3 sequence-specific 100BP primers were used to create patient integrase amplicons (2).
- The 100BP primer successfully amplified 53% (7 of 13 - not shown) of patient amplicons and only 29% of INSTI resistant amplicons (2 of 7- not shown) and thus a 25BP primer set was used as backup.
- Comparison of growth curves of recombinant integrase viruses shows that the viruses made with 100BP primers reached harvesting (25% GFP) 1-3 days earlier than viruses made with 25BP primers.



Reproducibility

- IC50 for NL4-3 virus over 5 independent experiments for 3 INSTI (RAL, EVG, DTG) were compared.
- Error bars represent 1 standard deviation from the mean of 5 experiments.





Patient INSTI resistance comparison

- Fold change (FC) generated by our HIV INSTI phenotype assay matched HIVdb (3) resistance predictions for these two patients with one exception- Patient 4 sample G below.
- Genotypes for this virus sample predicted high level resistance to RAL and EVG (HIVdb = 5; not shown) which correlated with phenotypic resistance FC >100.
- HIVdb predicted moderate resistance to DTG for this sample (HIVdb = 4) while our phenotype assay predicted susceptibility to DTG (FC = 1.8)

		Patient 2				Patient 4			
Samples	**pNL4-3**	**A Pre-RAL (2008)**	**B On RAL (2012)**	**C On DTG (2013)**	**D On DTG (2014)**	**E Pre-RAL (Oct. 2006)**	**F On RAL (Aug.2010)**	**G On RAL (Nov.2010)**	**H On DTG* (Oct.2014)**
Fold Change relative to pNL4-3 RAL / DTG / EVG	1.0 **1.0** 1.0	0.5 **1.1** 1.0	>100 **7.1** >100	>100 **>100** >100	>100 **>100** >100	2.0 **2.2** 4.8	20.5 **1.4** >100	>100 **1.8** >100	N/A
Predicted DTG resistance level (HIVdb)	1 Susceptible	1 Susceptible	4 Intermediate resistance	5 High-level resistance	5 High-level resistance	1 Susceptible	2 Susceptible	4 Intermediate resistance	N/A
Major integrase mutations	none	none	Q148H, G140S	Q148H, T97A, G140S	Q148H, L74M, T97A, G140S	none	N155H	Q148H, G140S	N/A
Viral Load (copies/mL)	N/A	2000	1400	800	4400	53000	61000	290000	40

*Patient 4 October 2014 sample H failed to amplify integrase amplicon due to low viral load

Patient sample oligogenome amplification sequence comparison

- DTG FC of sample B (above) of Patient 2 is 6.5-fold higher than pre-therapy sample A whereas FC for sample G (above) of Patient 4 is in the range of the pre-therapy sample E, despite similar mutations.
- This difference in FC may be explained by the T97A mutation, which in sample C is associated with a >100 FC. This is in line with the T97A resistance pathway previously established (1).
- Sequencing of samples B and G did not show the T97A mutant.
- Dilution of sample B was not done since the viral load was low (1400) possibly explaining why no T97A variants were found and indicating the limits of this technique.

	Successful amplifications	Number of variants	Number of unique sequences	Major mutation frequency
Sample B	**9/24**	**1**	**4**	**Q148H (9/9) , G140S (9/9)**
Sample G	**71/94**	**3**	**50**	**Q148H (67 of 68), G140S (67 of 68), N155NH (1 of 68), 1 variant with with no major mutations**

Conclusion

- Our in-house integrase phenotype assay can be used to generate IC50 for INSTI drugs.
- Oligogenome amplification produces integrase resistant virus variants that can be individually phenotyped and sequenced. These variants may be helpful in identifying rare INSTI resistant pathways not yet known.

Future Work

- Matched phenotype and genotype data and virological outcomes will be used to populate an INSTI database for the prediction of INSTI susceptibility.

Citations

- 1. Johnson VA, et al. 2013. Special Contribution. Update of the Drug Resistance Mutation in HIV-1: March 2013. Topics in Antiviral Medicine. 21(1):9
- 2. Brockman MA, et al. 2012. Uncommon Pathways of Immune Escape Attenuate HIV-1 Integrase Replication Capacity. J. Virol. 86(12):6914
- 3. http://hivdb.stanford.edu/index.html

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Conflict of Interest Disclosure: We have no conflicts of interest

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