Susceptibility to Bictegravir and Cabotegravir and Integration site preferences of HIV-1 non-B subtype Viruses from patients failing Raltegravir in Uganda

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Background

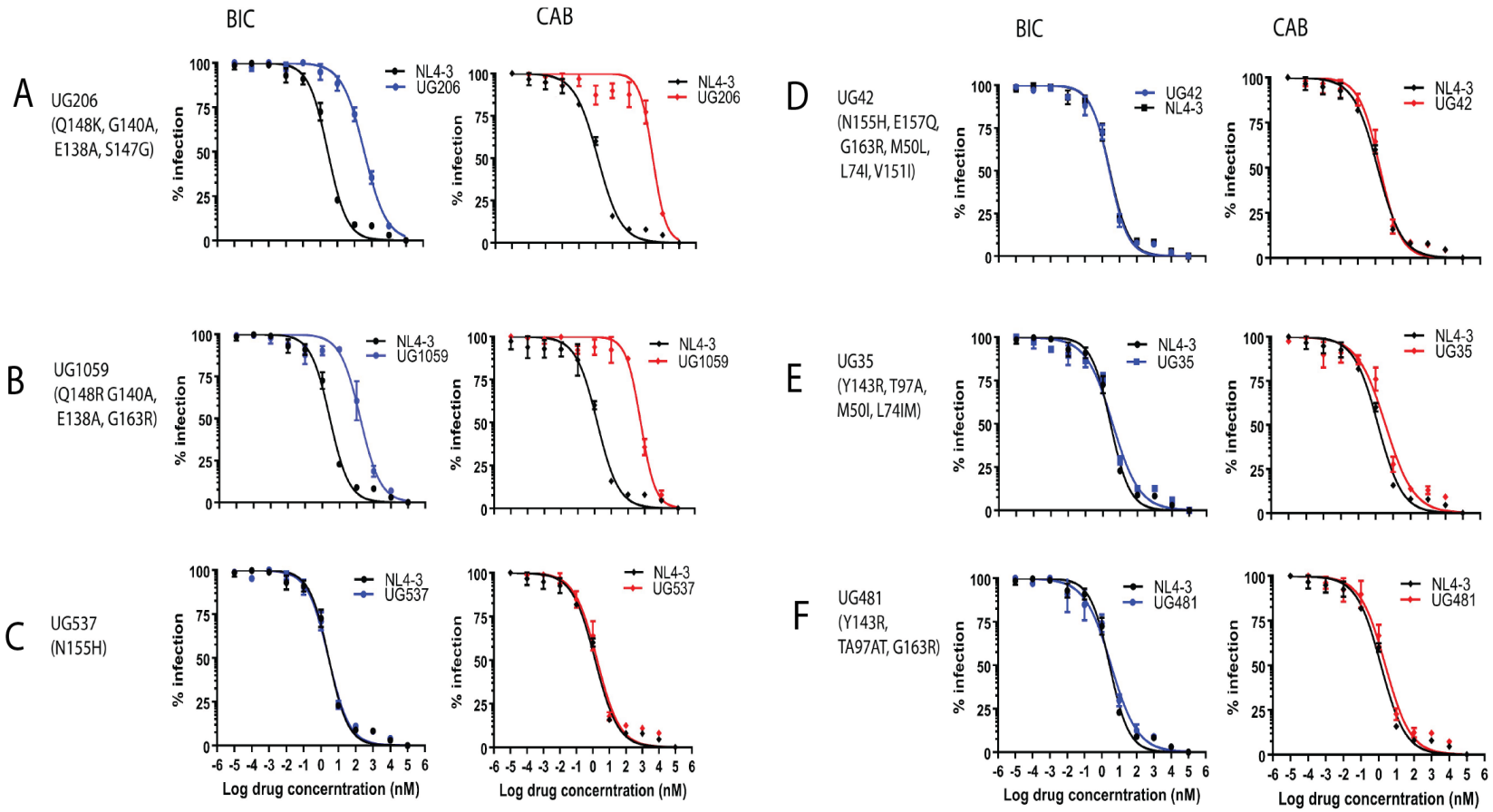
- Second generation integrase strand transfer inhibitor (INSTI) cabotegravir (CAB) was recently approved is long-acting injectable, and bictegravir (BIC) is becoming accessible in settings with high HIV-1 non-B subtype viruses.
- Data on impact of INSTIs drug resistance mutations (DRMs) on integration site preference and susceptibility to BIC and CAB remains very scarce especially in HIV-1 non-B subtypes.

Methods

- Phenotypic assays on HIV-1 integrase recombinant subtype A and D viruses from 8 patients failing RAL-based third-line in Uganda was done in TZM-bl cells. Drug resistance was expressed as fold change (FC) in effective concentration 50 (EC_{50}) between HIV-1 controls and integrase-recombinant viruses
- HIV-1 integration capacity into human genome was assessed in MT4 cells using Alu-gag qPCR.
- Integration site profiles were analyzed using total genomic DNA from HIV infected Ugandan patients: antiretroviral therapy (ART) naïve (n=30), raltegravir failing (n=30) and protease inhibitor failing patients (n=30) using Illumina MiSeq sequencing.

Results

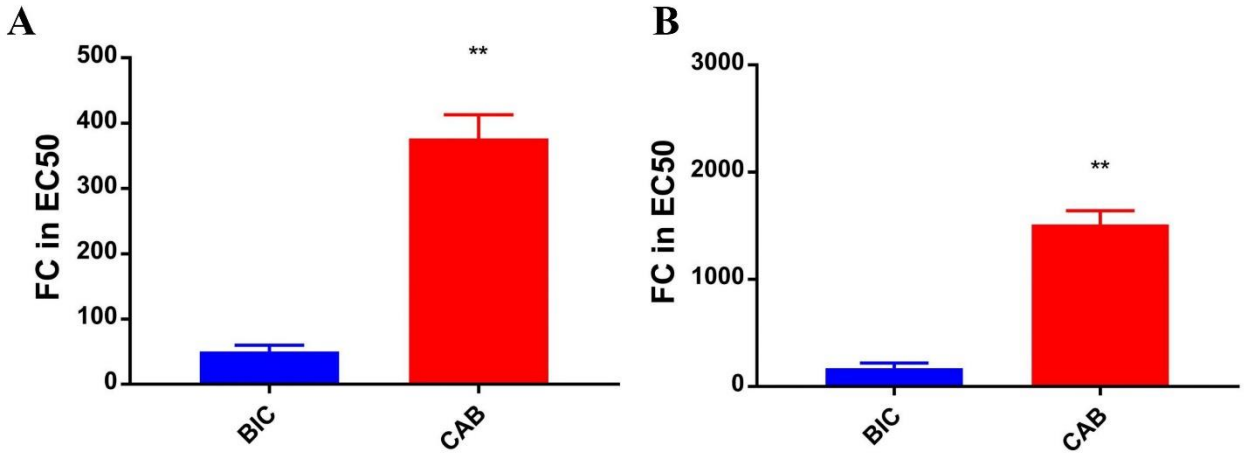
- HIV-1 integrase recombinant viruses harboring single N155H or Y143R/S mutations or in combination with secondary INSTIs mutations were susceptible to both BIC and CAB.
- Multiple primary INSTIs DRMs in form of E138A/G140A/G163R/Q148R, and E138K/G140A/S147G/Q148K mutations led to increased fold-change in EC_{50} to both CAB (FC, 429->1000) and BIC (FC, 60->100).



The susceptibility of recombinant viruses to CAB and BIC. Panel A- UG206, B- UG1059, C- UG537, D- UG42, E- UG35, and F- UG481, drug susceptibility to BIC (left panel) and CAB (right panel) respectively

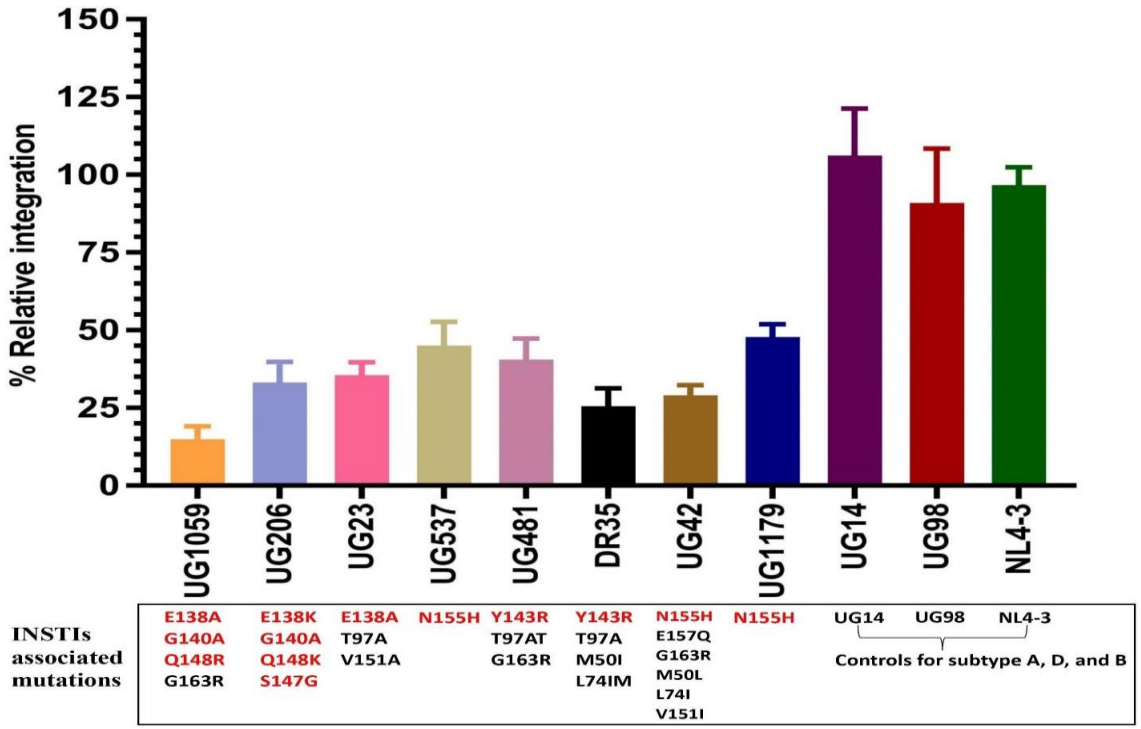
Results

- The reduction in drug susceptibility in presence of multiple primary INSTIs DRMs was significantly high with CAB compared to BIC ($P < 0.0023$).



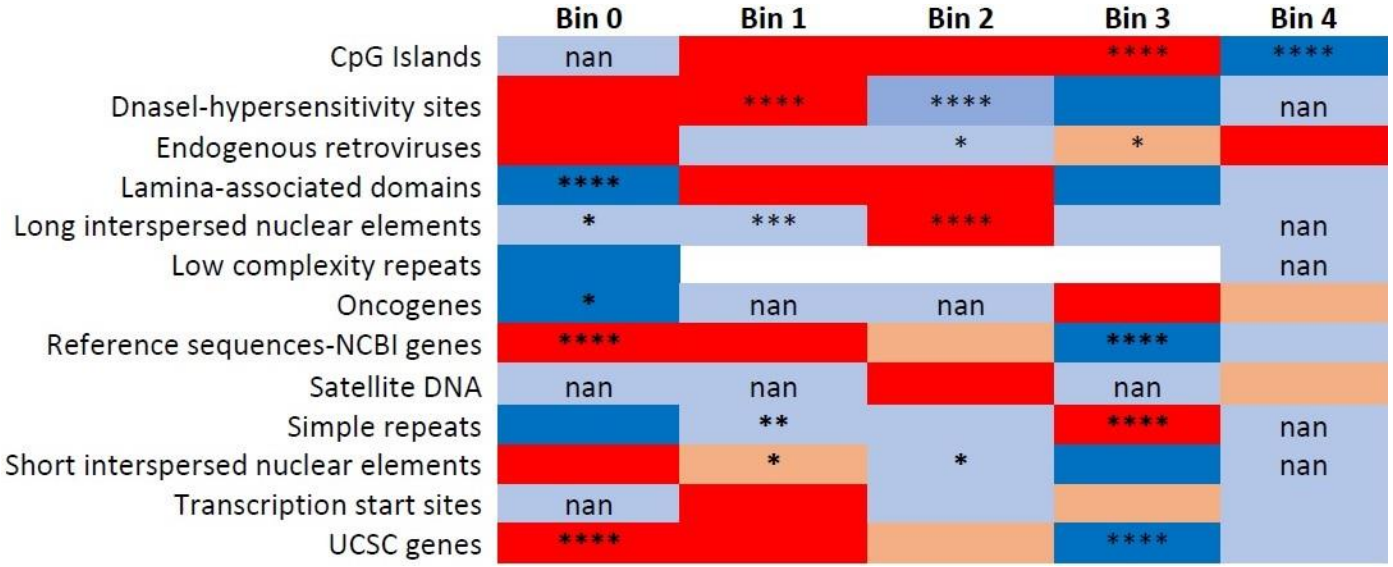
The fold-change in EC_{50} of recombinant viruses carrying multiple primary INSTIs-resistance mutations. A) the fold-change (FC) in EC_{50} (nM) of BIC and CAB for recombinant virus UG1059 (E138A/G140A/G163R/Q148R), B) UG206 (E138K/G140A/S147G/Q148K)

- Recombinant viruses showed impaired integration capacity, (<50%) relative to the wild type and controls.



The relative integration capacity of IN-recombinant viruses with diverse INSTIs-resistance mutations. The relative integration capacity of mutant viruses compared with controls (UG14 and UG98) and wild type (NL4-3) was determined in MT4 cells. The integrated HIV-1 LTR was amplified and quantified using (Alu-gag) qPCR. Means and ± SD are shown from two independent experiments carried out in triplicates for each sample. qPCR results were normalized relative to NL4-3 wild type arbitrary set at 100%.

Results and conclusions



Heatmaps depicting the fold enrichment or depletion of integration sites near common genomic features compared to matched random controls. Darker shades represent higher fold-changes in the ratio of integration sites to matched random control sites. Bins represent the distance of the integration sites from each genomic feature. Bin 0 = within the feature; Bin 1= 1-499 bp; Bin 2 = 500-4,999 bp; Bin 3 =5,000-49,999 bp; Bin 4 = >49,999 bp. Stronger relationships between retroviral integration site profiles are indicated by darker blue color in the pairwise distance matrix. Significant differences are denoted by asterisks (*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. Not a number (nan), 0 integrations were observed and 0 were expected by chance.

- Contrary to ART naïve, viruses from RAL failing patients with INSTIs DRMs significantly integrated into lamina associated domains ($P < 0.0001$) and oncogenes ($P < 0.05$).

Conclusions

- Single N155H or Y143S/R or in combination with secondary mutations, remain susceptible to both BIC and CAB, however, multiple primary INSTIs DRMs leads to increased resistance to CAB and BIC in HIV subtype A and D viruses. BIC and CAB offer alternative option to ART experienced patients. INSTIs DRMs may encourage formation of latent reservoirs and malignancies in patients failing raltegravir.