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Ex vivo antiretroviral potency of newer integrase strand transfer inhibitors cabotegravir and bictegravir in HIV-1 non-B subtypes

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Abstract

Objective—To determine the antiretroviral activity of the integrase strand transfer inhibitors (INSTIs), raltegravir, elvitegravir, dolutegravir, cabotegravir and bictegravir, against different subtypes as well as primary and acquired drug resistance mutations (DRM) in a patient-cohort infected with diverse subtypes.

Design—Biochemical and virological drug sensitivity analysis using patient derived HIV-1 genes and cross-sectional/longitudinal clinical study.

Methods—Assays for inhibition of 3'-end processing (IC_{50-3P}), strand transfer (IC_{50-ST}) and drug sensitivity (DSA) for five INSTIs were done using patient-derived *integrase* or *gag-pol* genes from subtypes A1, B, C, 01_AE and 02_AG. *Integrase* from INSTI-naïve (n=270) and experienced (n=96) patients were sequenced.

Results—Raltegravir had higher IC_{50-ST} than the other INSTIs for all subtypes. Elvitegravir had higher IC_{50-ST} for HIV-1C (p<0.05) and 02_AG (p<0.05) than HIV-1B. Dolutegravir showed lower IC_{50-ST} in HIV-1C than HIV-1B (p= 0.003). In CAB, the non-B subtypes showed lower IC_{50-ST} (p<0.05) than HIV-1B. In BIC, lower IC_{50-ST} in 01_AE (p=0.017) and 02_AG (p=0.045)

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UN and AS conceived and designed the studies and analysis plan. KS, LCR and SGS performed, analysed and supervised the biochemical data. SGA and DTN performed the virological assays supervised by UN. UN performed the data collection and sequence analysis. AS and BH interpreted the clinical data. UN wrote the first draft of the manuscript reviewed by KS and AS. All the authors approved the final version of the manuscript

than HIV-1B. In DSA, EC₅₀ for dolutegravir [median (IQR) 2.14 (1.3 to 2.56)], cabotegravir [1.68 (1.34 to 2.55)], and bictegravir [1.07 (0.22 to 2.53)] were lower than raltegravir and elvitegravir. One patient had a primary DRMs (0.3%, 1/270), but 17 (6.3%) had one major accessory DRM of which 12 were E157Q.

Conclusion—The equal or higher potency in non-B subtypes of dolutegravir, cabotegravir and bictegravir compared to raltegravir and elvitegravir confirms their suitability for use in countries dominated by non-B subtypes. Any impact of the high prevalence of major accessory mutations, especially E157Q, requires long-term follow-up studies.

Keywords

Integrase inhibitor; bictegravir; cabotegravir; resistance; non-B subtype

Introduction

Integrase strand transfer inhibitors (INSTIs) of the human immunodeficiency virus type 1 (HIV-1) have high potency, durable efficacy and low toxicity [1]. Three approved INSTIs, raltegravir (RAL), elvitegravir (EVG), and dolutegravir (DTG) have been approved by FDA and European Medicines Agency. INSTIs, bictegravir (BIC) and cabotegravir (CAB), are presently in phase III studies [2, 3]. The INSTIs DTG and BIC exhibits longer dissociation half-life in biochemical analyses of wild-type integrase/DNA complexes, resulting in a high genetic barrier to resistance [4, 5]. The high efficacy in clinical trials [6, 7] and in clinical care have prompted an increasing use of DTG in first-line antiretroviral therapy (ART).

The efficacy of the newer INSTIs (CAB and BIC) across various HIV-1 subtypes *in vitro* and *ex vivo* is however not well documented [8]. Similar to other antiretrovirals, the development of INSTIs was based to a large extent on clinical and virological data from HIV-1 subtype B (HIV-1B). Although, HIV-1 subtype has not been considered as a predictor for ART failure, an ACTG trial indicated HIV-1 subtype C (HIV-1C) as an independent predictor for viral treatment failure [9], similar to our study of the Swedish InfCare HIV cohort where a higher risk of viral failure was found in HIV-1C patients than in HIV-1B, despite developed clinical care, modern laboratory monitoring, and focused adherence support performed at highly HIV-specialised infectious disease clinics [10]. Also, subtype specific differences in drug resistance to first generation INSTIs have been reported [8] as well as in DTG cross-resistance pattern in patients failing RAL [11]. Further sequence and structure based analyses showed that the subtype-specific effects were caused by polymorphisms across the subtypes, which significantly affected native protein activity, structure, function of the drug-mediated inhibition of the enzyme [12].

The present study aimed to analyse if subtype specific differences exist between INSTIs (RAL, EVG, DTG, CAB and BIC) with regard to *in vitro* and *ex vivo* antiretroviral potency, using biochemical and virological assays. Furthermore, the presence of primary and major accessory drug resistance mutations (DRMs) as well as acquired DRMs was investigated in patients from Sweden in order to delineate any subtype-specific differences.

Material and methods

Cell lines, plasmids, and antiretrovirals

TZM-bl cells, plasmid pNL43, and RAL were obtained from the NIH AIDS Reagent Program. 293T cells were purchased from ATCC (Manassas, VA, USA). The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma, US), supplemented with 10% Fetal Bovine Serum and 2mM L-glutamine. EVG (Selleck-S2001) and DTG (Selleck-S2667) were purchased from Selleckchem (Selleck Chemicals, Germany). CAB and BIC were kindly provided by ViiV Healthcare/GSK and Gilead Sciences, respectively.

Clinical specimens and data

Stored plasma samples, used in the *ex vivo* studies, were randomly selected from 24 INSTI naïve adult patients from Stockholm, Sweden [10]. The viruses were pure subtypes (HIV-1B n=6; HIV-1C n=14; A1 n= 1) or circulating recombinant forms (CRFs) [01_AE (n=1); 02_AG (n=2)], as determined by near full-length sequencing using our earlier published protocol [13].

In addition, integrase sequences which had been obtained within routine clinical care through direct population sequencing of INSTI-naïve (n=270) or INSTI-experienced (n=96) patients were included for analysis of primary, major accessory or acquired DRM. Among the INSTI-experienced patients, 74% (71/96) were on RAL, 7% (7/96) on EVG, and 17% (16/96) on DTG, while two were experienced with both RAL and DTG. The resistance interpretation for RAL, EVG, and DTG were based on the Stanford HIV resistance database [14]. For CAB, the presence of E138A/Q148R, E138K/Q148K, E138K/Q148R, G140C/Q148R, G140S/Q148R, or Q148R/N155H was interpreted as high-level resistance [2]. If a virus contained only one of these mutations, it was assigned as potential low-level resistance. For BIC, the presence of E138K/Q148R, G140S/Q148R or N155H/Q148R was interpreted as high level resistance [3]. If a virus contained only one of these mutations, it was assigned as potential low-level resistance. Additionally, if the M50I/R263K mutation was present, we termed it as intermediate resistance to BIC [3].

Ethical permissions were obtained from the Regional Ethics Committee Stockholm (Dnr: 2006/1367-31/4 and 2014/928-31/2).

Cloning and purification of integrase

The complete coding region of the *integrase* genes (HXB2: 4230-5096) of four randomly selected INSTI-naïve patients infected by HIV-1B, C, CRF01_AE, and CRF02_AG, respectively, were cloned into the pRSFDuet-integrase vector with N-terminal H₆-tag. Recombinant integrase proteins were expressed in BL21-DE3-RIL cells. The expression of the IN protein was induced by addition of 1 mM IPTG in incubator-shaker for 3 hours at 37 °C. The cells were harvested and resuspended in 20 mM Tris-HCl pH 8.0, 1 M NaCl, 4 mM 2-Mercaptoethanol (βME), 5 mM imidazole, 10 mM 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate hydrate (CHAPS), 1mM phenylmethylsulfonyl fluoride (PMSF) and 0.15 mg/ml lysozyme. The homogenized and sonicated cells were centrifuged and the IN proteins were purified by Ni-affinity chromatography.

Inhibition of 3'-end processing assay (IC_{50-3P})

To assess the 3'-end processing activity of IN in the presence or absence of INSTIs, a 21-mer oligonucleotide was annealed to a complementary 5-³²P-labeled 21-mer oligonucleotide that contained a 3'-end-CAGT sequence (Table 1). The endonucleolytic cleavage of 3'-end GT, resulting in an exposed CA-dinucleotide at the 3'-end, was conducted by incubating 300 nM IN, 2 nM DNA substrate, in a buffer containing 20 mM MOPS, pH 7.2, 25 mM NaCl, 1 mM MnCl₂ and 3 mM βME. The cleavage products were resolved on a 16% polyacrylamide 8 M urea gel. The cleavage of the GT-dinucleotide resulted in a 19-mer product. The reactions were allowed to proceed for different times and stopped by adding of Sanger's gel loading solution (95% formamide, 0.01% bromophenol blue, 0.01% xylene cyanol, and 25 mM ethylenediaminetetraacetic acid). The fraction of cleaved GT-dinucleotide was calculated by measuring the 19-mer products, which were then plotted as a function of time. A time point representing the linear range of 3'-end processing reaction (90 minutes) was selected, and the 3'-endonuclease activity of INs was monitored with increasing concentrations of INSTIs (0 – 100 nM). The gels were exposed to PhosphoImager (GE Healthcare, USA), scanned on Typhoon FLA 700 (GE Healthcare, USA) and the radioactive bands were quantitated by ImageQuant 5.2 (GE Healthcare, USA). The amount of 3'-end excision products (19-mer) was plotted as the function of INSTI concentration, and the data were fit to a dose-response curve to estimate IC_{50-3P} (three replicates).

Inhibition of strand-transfer activity assay (IC_{50-ST})

The inhibition of strand transfer activity in the presence INSTIs was assessed in gel-based assay. This assay separates the products of the strand-transfer reaction, which are longer than the 5'-³²P-labeled 19-mer substrate. Briefly, a ³²P-5'-end-labeled 19-mer single-stranded DNA (ssDNA) was annealed to a complementary 21-mer ssDNA. The 19-mer substrate has a CA-dinucleotide exposed for the INST reaction. Three hundred nM IN was first incubated with 2 nM ³²P-5'-end-labeled 19-mer for 15 minutes followed by addition of 500 nM unlabelled 31 base-pair long double stranded DNA (dsDNA) and 1 mM MnCl₂. The reaction was allowed to proceed for 90 minutes in the presence of increasing concentrations of INSTIs (0 nM to 25 nM) followed by stopping by Sanger's gel loading solution. The products were resolved on a 16% polyacrylamide 8 M urea gel. The gels were exposed to PhosphoImager (GE Healthcare, USA), scanned and quantitated as described above. The radioactive bands corresponding to products of greater than 19-mer were results of the IN strand-transfer reaction. These bands were quantitated and plotted as the function of INSTI concentration. The data were fit to a dose response curve to determine IC_{50-ST} of IN proteins for strand-transfer reaction (three replicates).

Cloning and recombinant virus production

HIV-1 RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, US) from 140 µl of patients' plasma. The cDNA was generated using the SuperScript® III RT enzyme (Invitrogen, Life Technologies, MA, USA) with gene specific primer 6231R, as described by us (Thermo Scientific) [15]. The first-round PCR was performed using the high-fidelity KAPA HiFiHotStart Ready Mix (2x) (KAPA Biosystem, MA, USA) with primers 0682F and 6231R primers. The second-round PCR was performed using 0702F-*Bss**HIII* and 5798R-

SalI which has *BssHII* and *SalI* restriction sites, respectively. The amplified product was restriction digested followed by gel purification using the QIAquickGel Extraction Kit (Qiagen, USA). The *gag-pol* fragment (HXB2:0702-5798) was cloned in pNL43 *gag-pol* plasmid following digestion with *BssHII* and *SalI* (New England Biolab, US) and ligation with T4 DNA ligase (New England Biolab, US). The recombinant viruses were produced by transfecting the plasmids into the 293T cell line using FuGENE® HD Transfection Reagent (Promega, US). All the molecular clones were sequenced bi-directionally. No primary DRM was observed in any of the clones. M50I was present in seven of the sequences from the HIV-1B (n=3) and the HIV-1C (n=4) strains.

Drug sensitivity assay

The drug sensitivity assay (DSA) was done by determining to which extent the five INSTIs inhibited the replication of our 24 recombinant viruses in the TZM-bl cells. Briefly, serial dilutions of the drugs spanning from 10 μ M to 0.0001 μ M were added in triplicate in 96-well plates in complete DMEM media containing TZM-bl cells, followed by infection with a reference virus (NL43) or the corresponding patient derived recombinant viruses (HIV-1B: n=6; HIV-1C: n=14; HIV-1A1: n=1; HIV-1CRF_01AE: n=1; HIV-1CRF_AG: n=2) at a multiplicity of infection (MOI) of 0.05 IU/cells in the presence of 10 μ g/ml concentration of diethylaminoethyl (DEAE). Virus replication was quantified by measuring *Renilla* luciferase activity (relative light units [RLU]) using *Bright-Glo™ Luciferase* Assay System (Promega, US). Drug concentrations required for inhibiting virus replication by 50% (EC₅₀) were calculated by a dose response curve using nonlinear regression analysis (GraphPad Prism, version 5.01; GraphPad Software, La Jolla, CA). Each assays were performed using two to four biological replicates in triplicate.

Statistical analysis and data visualisation

Statistical analysis was performed using an in-house R script or in GraphPad Prism, version 5.01 (GraphPad Software, La Jolla, CA). The clustering analysis of the EC₅₀ fold-drug change of the patients derived recombinant viruses against the control virus NL4-3 was performed using QluCore Omics Explorer version 3.2. Comparisons of the EC₅₀ were conducted by the either Wilcoxon signed-rank test (for paired data), student t-test and Mann Whitney U test (for unpaired data) (between two groups) or analysis of variance (ANOVA) using the Kruskal–Wallis test by ranks (among three groups) where appropriate. A P value <0.05 considered significant.

Results

In vitro inhibition of strand transfer (IC_{50-ST}) and 3'-end processing activities (IC_{50-3P})

The IC_{50-ST} for strand transfer inhibition of RAL, EVG, DTG, CAB, and BIC are shown in Figure 1A. RAL had higher IC_{50-ST} than the other INSTIs in all subtypes (p<0.001). The drug specific comparison showed that RAL had comparable potency (IC_{50-ST}) for 01_AE and 02_AG but better potency for HIV-1C (p=0.047) when compared to HIV-1B. In contrast, EVG had relatively higher IC_{50-ST} for HIV-1C (p=0.016) and 02_AG (p=0.02). DTG showed lower IC_{50-ST} for HIV-1C than HIV-1B (p=0.003). For CAB, the non-B subtypes showed lower IC_{50-ST} (p=0.043, 0.027, and 0.026 for HIV-1C, 01_AE and 02_AG,

respectively) compared to HIV-1B. For BIC, no difference was found between HIV-1B and HIV-1C but a lower IC_{50-ST} was found for 01_AE and 02_AG ($p=0.017$ and 0.045 respectively) compared to HIV-1B.

INSTIs also inhibit 3'-end processing activity albeit at a lower efficiency than strand transfer activity. Hence, we determined IC_{50-3P} for all the IN against all the drugs (Fig. 1B). All INSTIs had a ~10-fold higher IC_{50-3P} for the 3'-end processing reaction compared to the strand transfer reaction.

Drug sensitivity assay

The *ex vivo* DSA was performed using recombinant HIV-1B ($n=6$), HIV-1C ($n=14$), and A-like (A1, 01_AE and 02_AG; $n=4$) viruses, derived from INSTI naïve patients (Fig. 2A). When the results of all recombinant viruses were analyzed, the EC_{50} for RAL and EVG [RAL: median (IQR): 5.31 (1.56–6.70)]; EVG: 2.82 (1.69–5.39)] were significantly higher ($p<0.05$, for all comparisons) than those for DTG, CAB, and BIC [DTG 2.14 (1.3–2.56); CAB 1.68 (1.34–2.55); BIC 1.07 (0.22–2.53)]. The EC_{50} of BIC were lower than for DTG ($p=0.01$). No difference was observed between BIC and CAB. Clustering analysis of the fold-change values identified specific clustering for RAL and EVG vs. other INSTIs, but not any subtype specific clustering (Fig 2B). While performing ANOVA among the group (Fig 2C–G), no statistical significance has been observed among the groups in RAL (Fig 2C), EVG (Fig 2D), CAB (Fig 2F) and BIC (Fig 2G). However, in DTG there was statistical difference as A-like viruses gave better *ex vivo* potency than HIV-1B ($p=0.019$) and HIV-1C ($p=0.011$) (Fig 2E).

Analysis of the integrase sequences from the patient cohort

The presence of primary integrase DRM was analyzed in 270 INSTI-naïve and ART experienced individuals, infected with HIV-1B ($n=92$), C ($n=82$), 01_AE ($n=25$), 02_AG ($n=15$), A1/A2 ($n=22$), other pure subtypes ($n=13$), and recombinant forms (CRFs or URFs) ($n=21$). One HIV-1B patient had a major primary INSTI-DRM, T66I (Supplementary Digital Content 1). In addition, 17 of 270 (6.3%) patients had at least one major accessory DRM: T97A ($n=3$), E157Q ($n=12$), or A128T ($n=2$). The A128T is a non-polymorphic mutation selected *in vitro* by EVG, but does not appear to reduce INSTI susceptibility *ex vivo*. The naturally occurring polymorphism M50I was found in 49 (18%) of the INSTI-naïve patients. None of the INSTI-naïve subjects had NRTI mutations; however, three individuals had NNRTIs DRM (V106M, K103N, G190A).

Thirteen of the 17 INSTI-naïve patients initiated an INSTI-based regimen with either DTG ($n=11$) or RAL ($n=2$). Among them seven initiated 2 NRTI + DTG OD, six switched from a 2 NRTI + 1 NNRTI regimen to an INSTI containing ART (DTG OD $n=3$; DTG BID $n=1$) with either abacavir/lamivudine or tenofovir disoproxil/emtricitabine. The reasons for switching the regimen was either failing on 2 NRTI + 1 NNRTI therapy ($n=4$) or due to side effects with undetectable viremia ($n=2$). The patients who failed an NNRTI ($n=4$) based regimen had one to three regimens prior to initiation of the INSTI. Among the NNRTI failure patients, two patients had K103N and V106M/Y188CH mutation respectively. No NRTI or PI mutations were found. The HIV-1 RNA load was also checked after six months.

Two RAL treated patients with E157Q and T97A mutations, respectively, failed to suppress viremia at month six. Eight of the nine patients on DTG, who had a major accessory mutation just before switch, showed low level viremia at month 6. One of the two patients who switched with suppressive viremia, maintained undetectable viral load, but one (with T97A) had a viral blip at month 6 (57 copies/mL) (Fig 3a). Sequencing of samples collected 1216 and 1038 days earlier from these two patients showed that they had E157Q and the T97A mutations, respectively. Taken together, 11 of 13 INSTI-naïve patients with INSTIs DRM before initiating RAL or DTG did not reach undetectable viremia after six months.

Among the INSTI-experienced patients failing on ART (n=96), 24% (23/96) had major INSTI DRM (Fig. 3b) and all of them had failed RAL. Four (17%) of the 23 patients were predicted to be fully susceptible to DTG, CAB, and BIC. Five patients (21%) with multiple mutations (Q148QR+N155H, G140A+Q148R, E138K+G140S+Q148R, E138K+G140A+S147SG+Q148K and G140S+Q148H+N155H) were predicted to have high-level cross resistance to DTG, CAB, and BIC, although no phenotypic assessment has confirmed this observation [2, 3]. The DRM profiles were similar in the HIV-1B and non-B subtypes.

Discussion

An increasing body of data suggest that the efficacy of antiretroviral therapy (ART) and the HIV-1 drug resistance pattern varies depending on the HIV-1 subtype. However, whether this holds true for second generation integrase strand transfer inhibitors (INSTIs) is not well-studied. Hence, this study was designed to evaluate the potency of INSTIs in different HIV-1 subtypes, using a combination of biochemical and cell culture assays. Overall, our data suggested that all of the three newer INSTIs (DTG, CAB, BIC) had a higher antiretroviral potency against HIV-1B as well as non-B subtypes compared to the older INSTIs (RAL and EVG), and no significant subtype specific difference in antiretroviral potency of the individual INSTIs was observed.

The integration of HIV DNA into host genome requires 3'-end processing and strand transfer reaction.[16] Similar to previous reports for RAL, EVG [17], DTG [18], and BIC [3], our data showed that the INSTIs are not as efficient in inhibiting 3'-end processing as they are in inhibiting strand transfer activity for all subtypes, as expected. It has been reported that 3'-end processing is as efficient in HIV-1 group O as in HIV-1 group M [19], however, to our knowledge no comparison among different HIV-1 subtypes has been done earlier. Our results suggest that the IC_{50-3P} for HIV-1C IN is highest among the tested subtypes for all INSTIs, except for BIC. Since the selectivity for strand transfer over 3'-end processing is driven by key enzyme-DNA interactions in the active site [18], it is possible that DNA/IN interactions in HIV-1C integrase differ from other subtypes. This interpretation could possibly be further supported by our results that the RAL IC_{50-ST} for HIV-1B and HIV-1A-like were significantly higher than the IC_{50-ST} of other INSTIs, but similar IC_{50-ST} were obtained for HIV-1C for all INSTIs. It must be pointed out here that the IC_{50-3P} values were determined with one integrase from four different subtypes. Hence, it is possible that these IC₅₀ values may change when several INs from different subtypes are cloned and inhibition assays are conducted. However, IC_{50-3P} values from our independently conducted assays for each subtype were consistent suggesting 'meaningful' differences.

In our DSA, we noted that DTG, CAB, and BIC had a higher potency than RAL and EVG independent of subtype, which is in line with recent studies on BIC [3] and CAB [2, 20]. Our cluster analysis also suggested that no differences existed in activity against the subtypes analysed for CAB, and BIC, although a better potency for A-like viruses were observed for DTG.

A natural polymorphism M50I was found in 18% of INSTI-naïve patients, which is in-line with the prevalence of this mutation in different subtypes in the Stanford HIV drug resistance database, ranging between 3% to 33% (median 10.5%) [14]. Mutation R263K mutation has been shown to confer 2- to 5-fold resistance to DTG, with decreased viral replication and strand transfer activity [21]. In breakthrough selections, M50I emerges after R263K [3] and provides replication advantage to R263K containing virus. A selection of R263K/M50I has been described in virus outgrowth assay for BIC that resulted 2.8-fold reduction of BIC-susceptibility, but M50I alone did not have any effect [3]. Seven of our 24 recombinant viruses had pre-existing M50I but the EC₅₀ for all INSTIs were very low confirming no effect of this mutation alone *ex vivo*.

While analyzing a large number of integrase sequences from INSTI naïve patients, we observed only one patient (0.4%) with a primary DRM. Instead, 17 (6.3%) patients had major INSTI accessory mutations (E157Q, T97A and A128T). These accessory mutations should not be interpreted as transmitted drug resistance and the importance of such natural polymorphisms is disputed. However, of 13 patients with these mutations, who were switched to RAL (n= 2) or DTG (n= 11) containing ART, only two had undetectable viremia after six months. Obviously, this is an anecdotal observation and other factors such as decreased adherence may also have contributed. However, the slow decline in viral load is still surprising to us, in view of the usual rapid decline when INSTIs are given and that the rate of undetectable viremia in the Swedish InfCare cohort is 95% after six months or more on ART [22].

In conclusion, our study showed that the three newer INSTIs (DTG, CAB, and BIC) had higher antiretroviral potency not only in HIV-1B but also in non-B subtypes compared to the first two approved INSTIs RAL and EVG. Moreover no significant difference among the newer INSTIs was seen with regard to their efficacy against different subtypes. Thus, DTG, CAB and BIC are attractive options for countries where non-B subtype infections are prevalent. Our analyses of patient derived sequences from INSTI-naïve individuals in clinical settings were reassuring with respect to the very low prevalence of primary INSTI DRM, despite the use of RAL and EVG since 2006. Furthermore, our sequencing results suggest that a relatively high rate of major accessory INSTI DRMs requires follow-up for their long-term impact on the outcome of INSTI-based ART.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. *In vitro* inhibition of strand transfer activity and 3'-end processing

A. 50% inhibitory concentrations (IC_{50-ST}) of strand transfer activity of RAL, EVG, DTG, CAB and BIC. Pair wise comparisons were done using t-test. **B.** 50% inhibitory concentrations (IC_{50-3P}) of inhibition of 3'-end processing of RAL, EVG, DTG, CAB and BIC. The data indicated that the inhibition of 3'-end processing was much weaker relative to the inhibition of strand transfer activity across subtypes. HIV-1 subtype B, subtype C, CRF01_AE, and CRF02_AG, were used.

Figure 2.

A. *In vitro* antiretroviral activity of INSTIs. Wilcoxon signed-rank test was used to perform pair wise analysis of the antiretroviral activity of raltegravir (RAL), elvitegravir (EVG), dolutegravir (DTG), cabotegravir (CAB), and bictegravir (BIC) on recombinant viruses obtained from INSTI naïve patients. The strains analysed were: HIV-1B (n=6) – pink; HIV-1C (n=14) – green; HIV-1A-like (A1, 01_AE and 02_AG; n=4) - maroon. The median (IQR) EC_{50} for the pNL4-3 control viruses were RAL: 3.23 (1.79 – 1.81), EVG: 1.96 (1.78 – 2.55), DTG: 2.05 (1.76 – 2.27), CAB: 2.47 (1.97 – 2.47) and BIC: 2.35 (1.76 – 2.53) using a TZMbl-cell assay. The bars represent the median value of three to eight biological replicates in triplicates. **B. Clustering analysis of the EC_{50} fold change of the patients derived recombinant viruses against the control virus NL4-3.** The analysis was performed using QluCore Omics Explorer version 3.2. Log₂ fold change values of each of the recombinant viruses compared to NL4-3 are depicted in red (+2, higher) to green (-2, lower) colour scale. **C–G. Subtype specific difference among the INSTIs.** C. Raltegravir, D. Elvitegravir (EVG), E. dolutegravir (DTG), F. cabotegravir (CAB), and G. bictegravir (BIC). The bar indicates analysis of variance (ANOVA) using the Kruskal–Wallis test by ranks while bracket indicates analysis by Mann Whitney U test.

Figure 3.

A. HIV-1 RNA load at initiation of INSTI containing combination therapy and after six months. Log transformed RNA copies/mL is presented for 11 INSTI-naïve patients with a major accessory mutation (T97A, A128T or E157Q), who initiated dolutegravir (DTG) containing triple therapy. **B. Drug resistance profile and level of resistance on patients failing raltegravir based therapy.** For RAL, EVG and DTG drug resistance mutations were identified according to Stanford HIV resistance database [14], while for CAB and BIC the mutations were identified as reported in the literature (see method section) [3, 20].