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Resistance and Cross-Resistance to First Generation Integrase Inhibitors: Insights from a Phase 2 Study of Elvitegravir (GS-9137)

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Introduction

- Human immunodeficiency virus (HIV) encodes three enzymes essential for viral replication: reverse transcriptase (RT), protease (PR) and integrase (IN)
- Currently approved antiretroviral (ARV) drugs, including NRTIs, NNRTIs and PIs inhibit the enzymatic activity of RT and PR
- Elvitegravir (EVG, GS-9137/JTK-303) is a potent inhibitor of the HIV IN strand transfer reaction (Serum-free antiviral IC_{50} = 0.2 nM; IC_{90} = 1.2 nM in PBMCs)[1]
- EVG is active against HIV-1 isolates resistant to all currently approved ARV drug classes and against HIV-2[1]
- EVG is undergoing a Phase 2 dose-ranging study (Study GS-US-183-0105) as a once-daily (QD), ritonavir-boosted therapy in ARV-experienced HIV-1 infected patients[2]
- Selection of HIV-1 variants with resistance to EVG *in vitro* has been shown to involve primary mutations at IN codons Thr66 (T66I) and Glu92 (E92Q)[3,4]
- The IN genotypes and phenotypes of HIV-1 from patients experiencing virologic failure on EVG-containing regimens (125 mg EVG boosted with 100 mg ritonavir, QD, EVG/r) in study GS-US-183-0105 are described

Objectives

- Characterize the IN genotypic patterns emerging in HIV-1 from patients experiencing virologic failure on 125 mg EVG/r-containing regimens through Week 24 of Study 0105
- Characterize the phenotypic susceptibility to EVG and MK-0518 (raltegravir, RAL) of HIV-1 isolates from patients experiencing virologic failure on EVG/r-containing regimens
- Characterize the impact of IN mutations on viral replication capacity

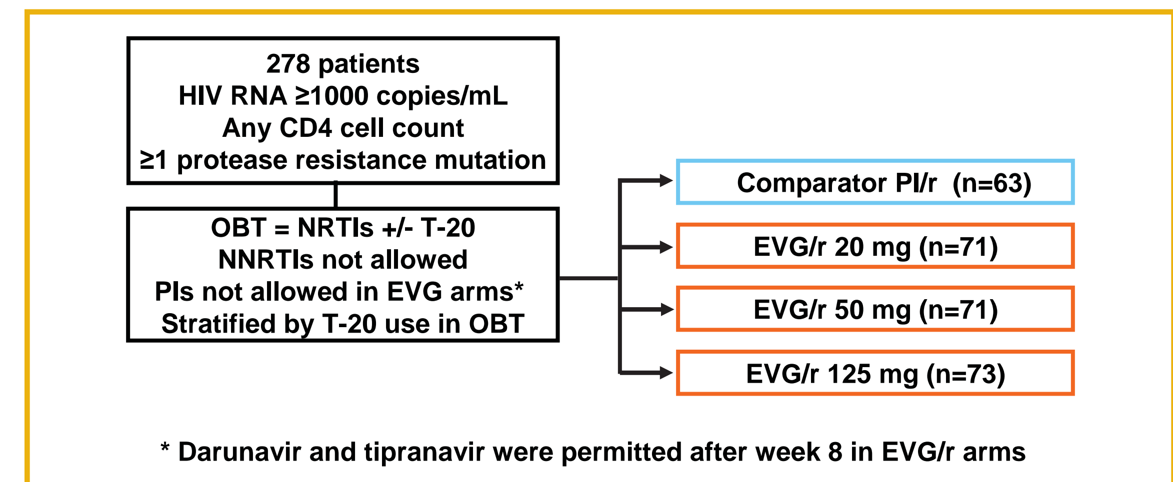
Methods

- **Study Design**: Study GS-US-183-0105 is an ongoing, randomized, active control, partially blinded (dose of EVG) dose-ranging 48 week Phase 2 study. The study is a non-inferiority study of ritonavir-boosted EVG (EVG/r) versus ritonavir-boosted comparator PI (CPI/r) in combination with an optimized background therapy (OBT) in ARV-experienced, HIV-1 infected patients. The OBT initially included only NRTIs +/-enfuvirtide (T-20) (used at the investigator's discretion). Patients were randomized 1:1:1:1 to one of three doses of EVG (20, 50 or 125 mg EVG, all boosted with 100 mg ritonavir) or to the CPI/r arm. The use of the PIs darunavir or tipranavir in the EVG/r arms was permitted after a protocol modification at week 8.
- **Virologic Failure (VF) Patients For Integrase Genotype and Phenotype Analysis:** Virologic failure (VF) was defined as HIV-1 RNA > 400 copies/mL and < 1 $\log_{10}$ reduction from baseline by week 12 confirmed at an unscheduled visit; OR, at any time after week 16, a confirmed rebound to < 1 $\log_{10}$ reduction from baseline and > 400 copies/mL HIV-1 RNA. Patients from the EVG/r 125 mg arm (n=30) meeting these criteria were analyzed for IN genotype and EVG and RAL phenotype at baseline and the confirmed VF visit. Data were obtained on 28 patients; 2 patients failed integrase genotype and phenotype analysis due to failure to amplify by RT-PCR.
- **PhenoSense INI Assay**: A 1611 base pair RHIN fragment containing the 3' end of RT (amino acids 317-560) and the complete IN coding region (amino acids 1-288) from the HIV-1 *pol* gene was amplified by RT-PCR of viral RNA from patient plasma. The patient derived amplicon was inserted into a genomic vector pHIVlucΔRHIN developed from an NL4-3 infectious clone, generating a pool of resistance test vectors (RTV). Pseudotyped virus stock was generated by co-transfecting HEK293 cells with RTV and AMLV *env* DNA that encodes the envelope protein from amphotrophic murine leukemia virus. Pseudotyped viruses were harvested from transfected cells and used to infect fresh HEK293 cells in the presence of serial dilutions of EVG and RAL, both synthesized at Gilead Sciences and provided in a blinded fashion to Monogram Biosciences. Quantification of luciferase activity in cells infected with pseudotyped virions in the presence of EVG and RAL was used to derive IC_{50} values for each drug against each patient derived virus. Results were expressed as fold-change in IC_{50} relative to the NL4-3 reference.
- **GeneSeq INI Assay:** The 1611 bp patient-derived fragment in the RTV pools was sequenced using 8 overlapping sequencing primers, providing double coverage for each nucleotide position. Sequence reactions were analyzed using an ABI3730 automated sequencer; chromatograms were aligned and edited using Sequencher software (GeneCodes, Ann Arbor, MI). Data were compared to a reference NL4-3 sequence.
- **IN Replication Capacity:** The contribution of the RHIN fragment to replication capacity (RC) was assessed by measuring luciferase activity in infected cells in the absence of drugs and normalized for transfection efficiency based on luciferase activity in transfected cells. RHIN RC of recombinant viruses derived from patient isolates was expressed as the percent replication of the NL4-3 reference. For PR-RT RC (as reported in the PhenoSense GT Assay) values are adjusted so that the median of drug susceptible viruses is approximately 100%. A similar adjustment factor was applied to the RHIN RC values. A RHIN-specific adjustment factor may eventually be required. Therefore, RHIN RC data reported here should be considered preliminary and interpreted with caution.

Methods (cont'd)

- **Construction of IN Site-Directed Mutants and Phenotyping:** The *Apa*1-*Sal*1 fragment of the HIV-1 *pol* gene in vector pUNV5-HisB was mutated (Quikchange, Stratagene) and transformed into PIR1 *E.coli* cells (Invitrogen). Plasmid DNA was purified (Qiagen), restriction digested with *Apa*1-*Sal*1, and the mutated *pol* fragment ligated to an HXB2 proviral vector and transformed into XL10-Gold *E.coli* cells. Positive clones were transfected into 293T cells; after 2 days incubation at 37°C viral pools were measured by p24 (Beckman-Coulter) and harvested. Viral RNA was extracted (Qiagen) and dsDNA prepared. Both strands of the IN coding region were sequenced to confirm the mutant genotype. For phenotyping, the MOI of mutant viruses was normalized to obtain a signal-to-noise ratio of 4-7 for uninfected versus infected cells in the absence of drug (chemiluminescent detection, Cell Titer-Glo, Promega). MT-2 cells were infected for 3 hours then incubated in triplicate drug dilution series for 5 days at 37°C. Cell viability data were converted to "% cell death"; antiviral IC_{50}s were determined using curve fitting (GraphPad, Prism).

Figure 1. Study GS-US-183-0105 Design



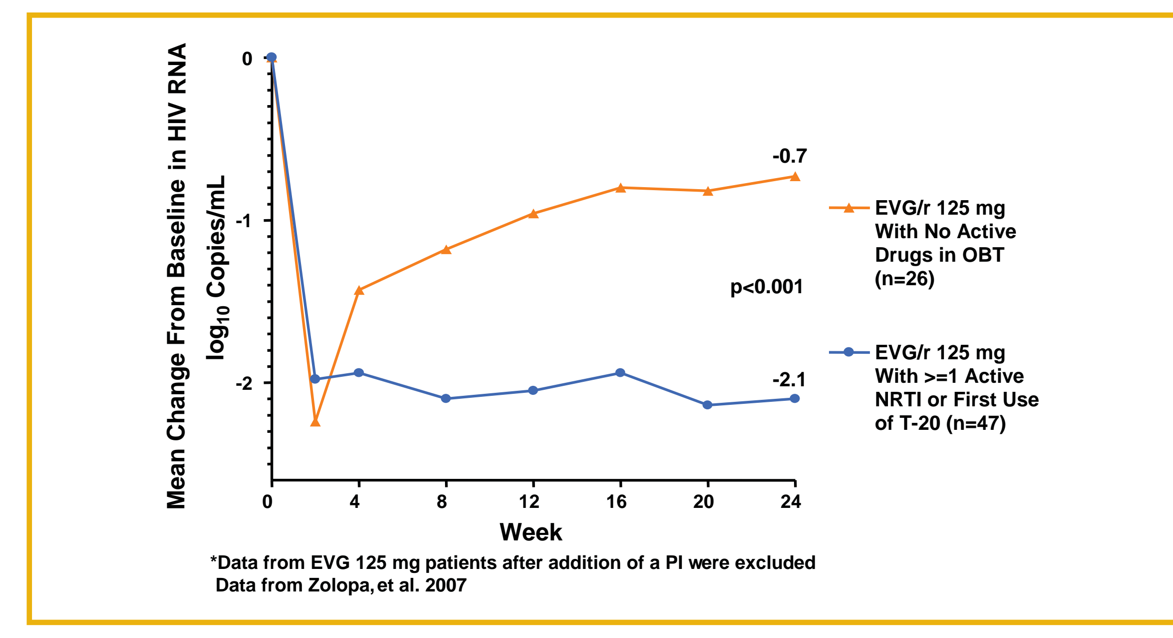
Results

Table 1. Study 0105 Baseline Characteristics[a]

Baseline Parameters	CPI/r n=63	EVG/r 20 mg, n=71	EVG/r 50 mg, n=71	EVG/r 125 mg, n=73
Mean HIV-1 RNA, $\log_{10}$ c/mL	4.54	4.66	4.47	4.71
Mean CD4 cells per mm^3	158	180	243	157
Genotypic Sensitivity Score, GSS=0 for all NRTIs in OBT[b]	32 (51%)	35 (49%)	34 (49%)	35 (48%)
PI Resistance Mutations, Median #	11	11	10	11
First Use of T-20	12 (19%)	12 (17%)	17 (24%)	19 (26%)
ARVs in OBT including T-20, Median #	3	3	3	3

a. Data from Zolopa, et al. 2007
b. GSS calculated using ANRS algorithm

Figure 2. Change from Baseline in HIV-1 RNA with EVG/r 125 mg: Influence of Activity of OBT*



*Data from EVG 125 mg patients after addition of a PI were excluded
Data from Zolopa, et al. 2007

Results (cont'd)

Figure 3. PhenoSense™ HIV for Integrase Inhibitors (INI)

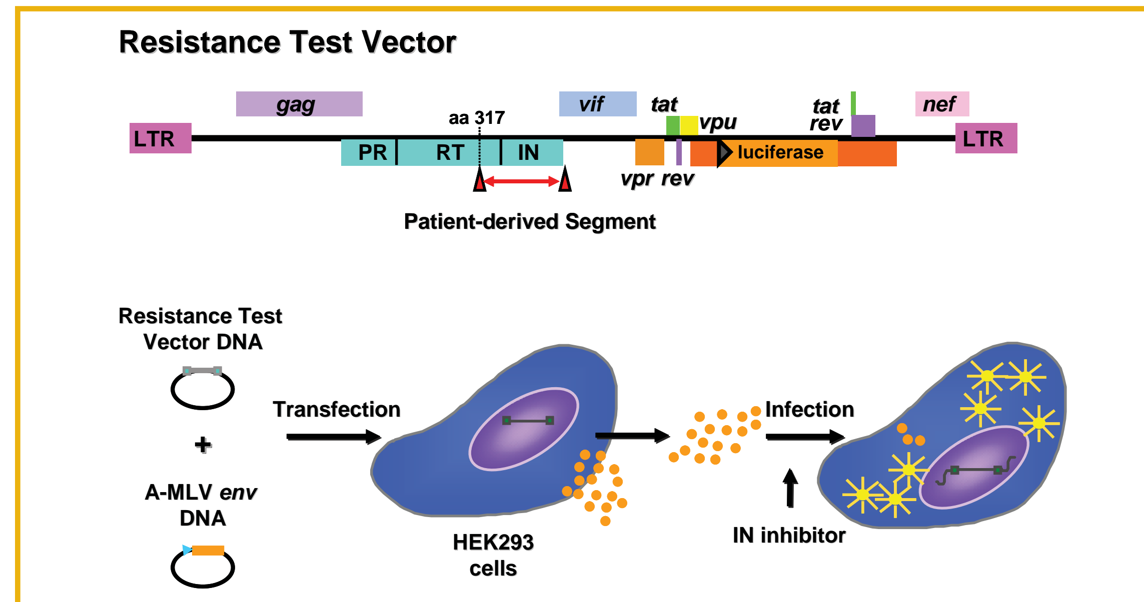


Figure 4. IN Mutations Developing in HIV-1 from >1 EVG/r 125 mg VF Patient By Week 24 (n=28)

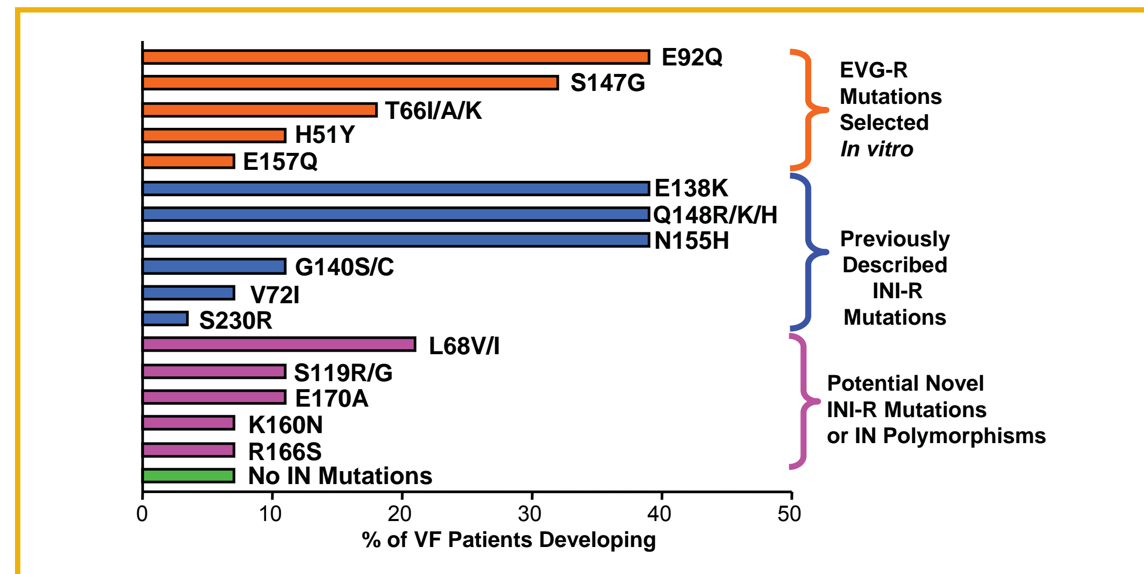


Table 2. IN Genotypic Patterns Developing in HIV-1 from EVG/r 125 mg VF Patients By Week 24 (n=28)[a]

IN Genotypic Patterns (population sequencing)[b,c]	N (%)
Any E92Q	11 (39%)
+ N155H (+/- other mutations)	4 (14%)
+ T66A (+ other mutations, no N155H)	3 (11%)
+ other mutations (no T66I/A/K, no N155H)	4 (14%)
Any N155H	11 (39%)
+ other mutations (no E92Q)	7 (25%)
+ T66I (+ other mutations, no E92Q)	1 (4%)
+ E138K (+ other mutations, no E92Q, no T66I)	3 (11%)
+ other mutations (no E92Q, no T66I, no E138K)	3 (11%)
E138K + S147G + Q148R (+/- other mutations)	6 (21%)
G140C/S + Q148R/H/K (+/- other mutations, inc T66K)	3 (11%)

a. Thirty patients were analyzed; 2 patients failed integrase genotyping for technical reasons
b. Median number of IN mutations developing: n = 4 (range 0-9)
c. Mutations were frequently observed as mixtures and may not be present on the same viral genome; some individual patients are classified in > 1 of the groups listed.

Table 3. EVG and RAL Phenotypes (PhenoSense INI Assay) of HIV-1 from EVG/r 125 mg VF Patients (n=28)

Phenotypic Parameters		Fold Change (Relative to NL4-3)
Baseline EVG:	Mean ± SD	1.50 ± 0.45
	Median (Range)	1.42 (0.91 - 2.53)
EVG at EVG VF:	Mean	> 151[a]
	Median (Range)	152 (1.02 - 301)
RAL at EVG VF:	Mean	> 28[b]
	Median (Range)	10.0 (0.78 - > 256)

a. For several viruses, fold change in IC_{50} values at VF to one or both drugs was reported as being above the limit of quantification in the PhenoSense INI Assay. Calculated mean and median fold change values at EVG/r VF are therefore estimates for both drugs.

Figure 5. Correlation of EVG and RAL Susceptibility Among EVG/r 125 mg VF Isolates (n=28)

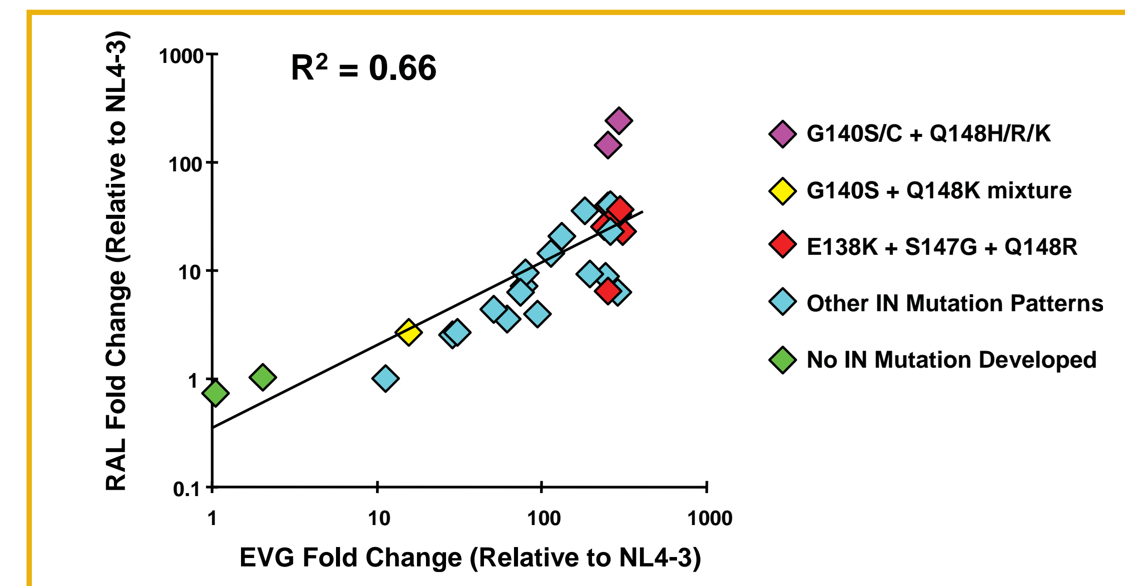


Table 4. EVG and RAL Phenotypes of Site-Directed Mutant HIV-1

Fold Change of Mutant Viruses: Single IN Mutations									
Drug	T66I	E92Q	E138K	G140S	S147G	Q148H	Q148K	Q148R	N155H
EVG	15	33	0.7	5.0	8.0	6.4	67	118	38
RAL	1.4	6.0	0.9	2.0	1.0	20	34	30	23
TFV	0.9	1.0	1.0	0.8	0.8	0.9	0.8	0.7	1.0
LPV	1.0	1.0	0.9	0.8	0.8	0.7	0.9	0.7	1.0

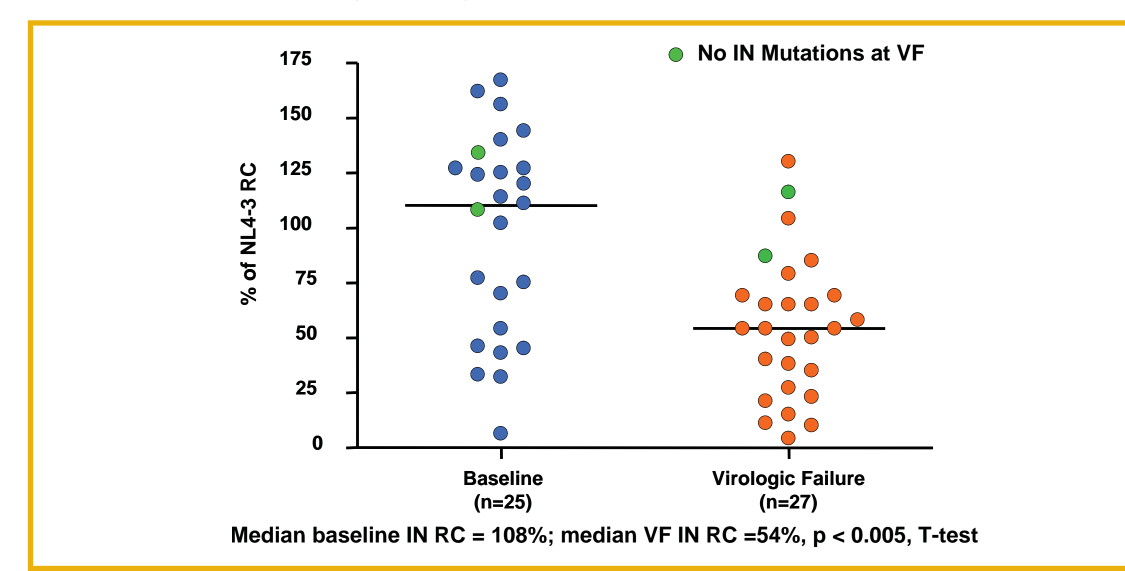
Fold Change of Mutant Viruses: Clinical EVG-R Mutation Patterns					
Drug	T66I S147G	T66I E92Q	E92Q N155H	G140S Q148H	E138K S147G Q148R
EVG	46	145	166	> 1000	175
RAL	2.5	33	135	> 1000	34
TFV	1.1	0.9	0.9	0.9	0.9
LPV	1.0	1.0	0.7	0.9	0.8

EVG: elvitegravir; RAL: raltegravir; TFV: tenofovir; LPV: lopinavir
Green: FC ≤ 2.5; Yellow: FC > 2.5 ≤ 10; Orange: FC > 10
All data based on at least n=3 independent experiments

Summary of Site-Directed Mutant Data

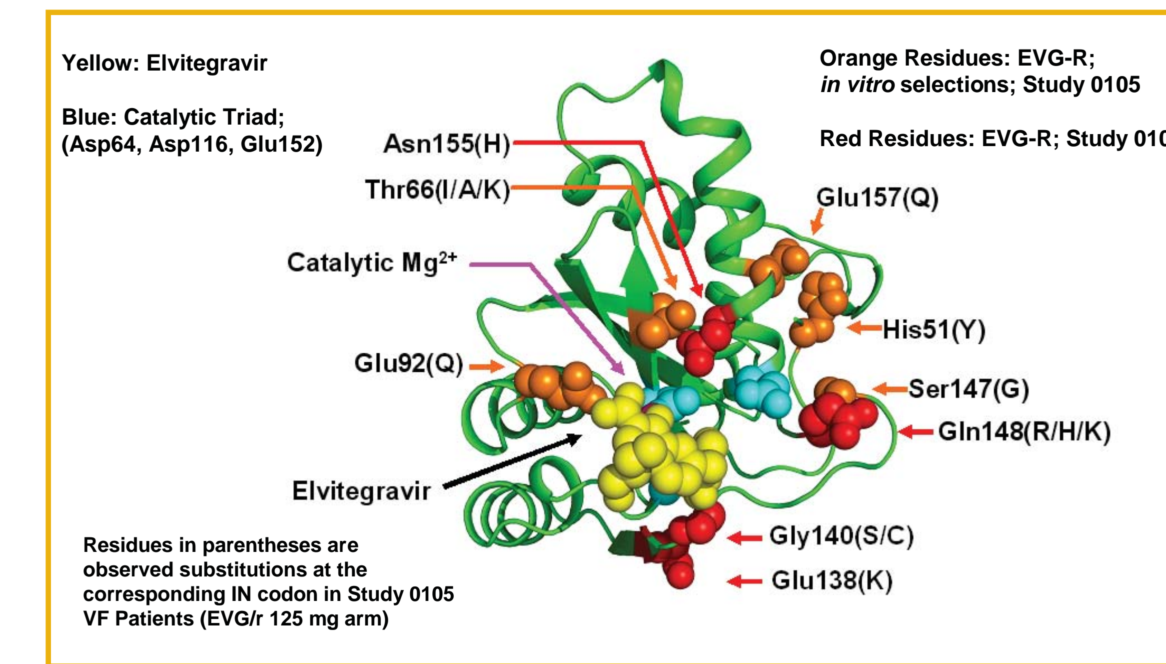
- Susceptibility of both EVG and RAL are reduced by E92Q, Q148H/K/R and N155H
  - These mutations were observed in both EVG/r and RAL clinical studies[5]
- Other mutations reduce susceptibility to one drug more than the other
  - T66I and S147G each reduce susceptibility to EVG > RAL
  - Q148H alone reduces susceptibility to RAL > EVG
  - E138K alone has no effect on susceptibility to either drug
- Combinations of IN mutations observed in some EVG/r VF patients reduce susceptibility to both EVG and RAL
  - G140S + Q148H: > 1000-fold reduced susceptibility to both INIs
  - E92Q + N155H: > 100-fold reduced susceptibility to both INIs
  - These patterns were also observed in RAL clinical studies[5]
- Some combinations with the T66I mutation, eg, T66I+S147G, retain susceptibility to RAL

Figure 6. IN Replication Capacity of Patient Isolates at Baseline and EVG/r 125 mg Virologic Failure in Study 0105



Median baseline IN RC = 108%; median VF IN RC =54%, p < 0.005, T-test

Figure 7. Model of Elvitegravir Bound to the IN Catalytic Core Domain and Residues Associated with Resistance



Conclusions

- **In highly treatment-experienced patients, use of 125 mg EVG/r QD and at least one additional active NRTI or T-20 resulted in a sustained 2 $\log_{10}$ reduction in HIV RNA through 24 weeks**
- **Among patients in the 125 mg EVG/r arm of Study 0105 with virologic failure, most developed IN mutations (median of 4 IN mutations)**
- **T66I/A/K, E92Q, E138K, S147G, Q148R/H/K and N155H were among the most common IN mutations observed (18% to 39% of VF patients)**
- **Common mutation patterns included E92Q + other mutations, E92Q + N155H, N155H + other mutations, E138K + S147G + Q148R and G140S/C + Q148R/H/K**
  - **These patterns overlap with the two major resistance patterns observed in raltegravir clinical trials (N155H + others; Q148K/R/H + others)**
- **Development of IN mutations was associated with reduced susceptibility to both EVG and RAL in many cases**
  - **Patterns containing E92Q + N155H, E138K + S147G + Q148R or G140S/C + Q148H/R mutations were associated with significantly reduced susceptibility to both drugs**
- **Development of IN mutations was associated with reduced viral replication capacity**

References

1. Matsuzaki, et al. 2006; Poster #508, 13th Conference on Retroviruses and Opportunistic Infections. Denver, CO.
2. Zolopa, et al. 2007, Oral Presentation 143LB, 14th Conference on Retroviruses and Opportunistic Infections. Los Angeles, CA.
3. Jones, et al. 2007; Poster 627, 14th Conference on Retroviruses and Opportunistic Infections. Los Angeles, CA.
4. Kodama, et al. 2006; Poster H-254; 46th Interscience Conference on Antimicrobial Agents and Chemotherapy. San Francisco, CA.
5. Cooper, et al. 2007; Oral 105AaLB, 14th Conference on Retroviruses and Opportunistic Infections. Los Angeles, CA.