

Virologic Outcome by V3 Loop Genotypic Population Sequencing and 454 “Deep” Sequencing in Clade B and Non-B Virus in MERIT at 48 and 96 Weeks

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Objective

- Using the original Trofile™ data set and using virological response as the gold standard, the aim of this study was to assess the performance of the Enhanced Sensitivity Trofile Assay (ESTA) and genotypic tropism determination by population sequencing and 454 “deep” sequencing to predict treatment response across the different HIV-1 subtypes (clades) in the MERIT treatment-naïve maraviroc (MVC) study

Background

- The Maraviroc versus Efavirenz Regimens as Initial Therapy (MERIT) study was designed to evaluate the efficacy and safety of MVC 300mg BID vs efavirenz (EFV), both in combination with fixed-dose zidovudine/lamivudine (ZDV/3TC), in treatment-naïve patients with CCR5-tropic (R5) HIV-1.¹
- HIV-1 co-receptor tropism was initially determined at screening by the original phenotypic Trofile assay (Monogram Biosciences), which was subsequently replaced by the ESTA
- A post-hoc analysis of MERIT screening samples demonstrated that both population and deep sequencing genotypic tropism tests predicted virologic response with equivalence to ESTA.^{2,3}
- A study in non-B HIV-1 subtypes showed that the Trofile assay performed well across subtypes, although it did need optimization for non-B subtypes. However, the ability to predict response to MVC across subtypes was not established.^{4,5}

Methods

- At study screening, HIV-1 subtype was determined for samples with a viral load ≥ 1000 HIV-1 RNA copies/mL, based on reverse transcriptase and protease gene sequence (Monogram Biosciences, South San Francisco, CA, USA)
- Virological response (viral load < 50 copies/mL) was assessed at weeks 48 and 96 for each rescreening tropism method for HIV-1 clade B, C, and other clades
- From the original Trofile R5 population, HIV-1 co-receptor tropism was determined phenotypically by the Trofile ESTA assay (Monogram Biosciences, South San Francisco, CA, USA), and genotypically by population or 454 “deep” sequencing
 - 454 “deep” sequencing
 - The V3 loop of HIV *env* was amplified in triplicate with nested RT-PCR and combined in equal quantities before sequencing using a 454/Roche GS-FLX
 - Population sequencing
 - Plasma samples were amplified in triplicate using RT-PCR and sequenced using an ABI 3730
 - Automated base-calling was performed using custom ReCall software without manual review
 - Genotype tropism predictions were made using the geno2Pheno (g2P) co-receptor algorithm with previously defined cut-offs
 - Tropism was inferred from V3-loop sequences, with samples classified as non-R5 if $\geq 2\%$ of the virus population scored ≤ 3.5 using the g2P algorithm. The population-based sequencing cut-off was 5.75 using g2P

Results

- A total of 360 subjects received MVC. Of these, 210 (58%) were infected with HIV-1 subtype B, 114 (32%) with subtype C and 36 (10%) with other subtypes
- Tables 1 and 2 show the baseline characteristics and subtype distribution of the MVC-treated population at randomization
- Figures 1-4 show virologic response as the percentage of patients with serum HIV-1 RNA < 50 copies/mL at weeks 48 and 96. Results are stratified by method of designating R5 tropism: ESTA, population-based V3 sequencing and deep sequencing

Table 1. Baseline Characteristics of Subjects in the MVC Arm of the MERIT Study by HIV-1 Subtype

	Subtype B N=210	Subtype C N=114	Other Subtype N=36
Male, n (%)	185 (88)	55 (48)	35 (15)
Prior AIDS diagnosis (Category C), %	6.2	21	11
Median CD4 ⁺ count, cells/mm ³ (range)	266 (6-966)	211 (12-793)	244 (4-557)
Mean HIV-1 RNA, log ₁₀ copies/mL	4.9	5.0	5.1

HIV-1 subtype was undetermined for one individual assigned to MVC

Table 2. Distribution of HIV-1 Subtypes in the Screened MVC Arm of the MERIT Study

HIV-1 Subtype	Prevalence n (%)
Screened Population	N = 360
Subtype B	210 (58.3)
Subtype C	114 (31.6)
Other Subtype	
BF	22 (6.1)
AG	6 (1.6)
A1	2 (0.56)
F1	2 (0.56)
A	1 (0.28)
D	1 (0.28)
F	1 (0.28)
Undetermined	1 (0.28)

Figure 1. Equivalent Virological Responses at Weeks 48 and 96 in the Original Trofile Population Rescreened R5 With ESTA, Population Genotyping and 454 Genotyping

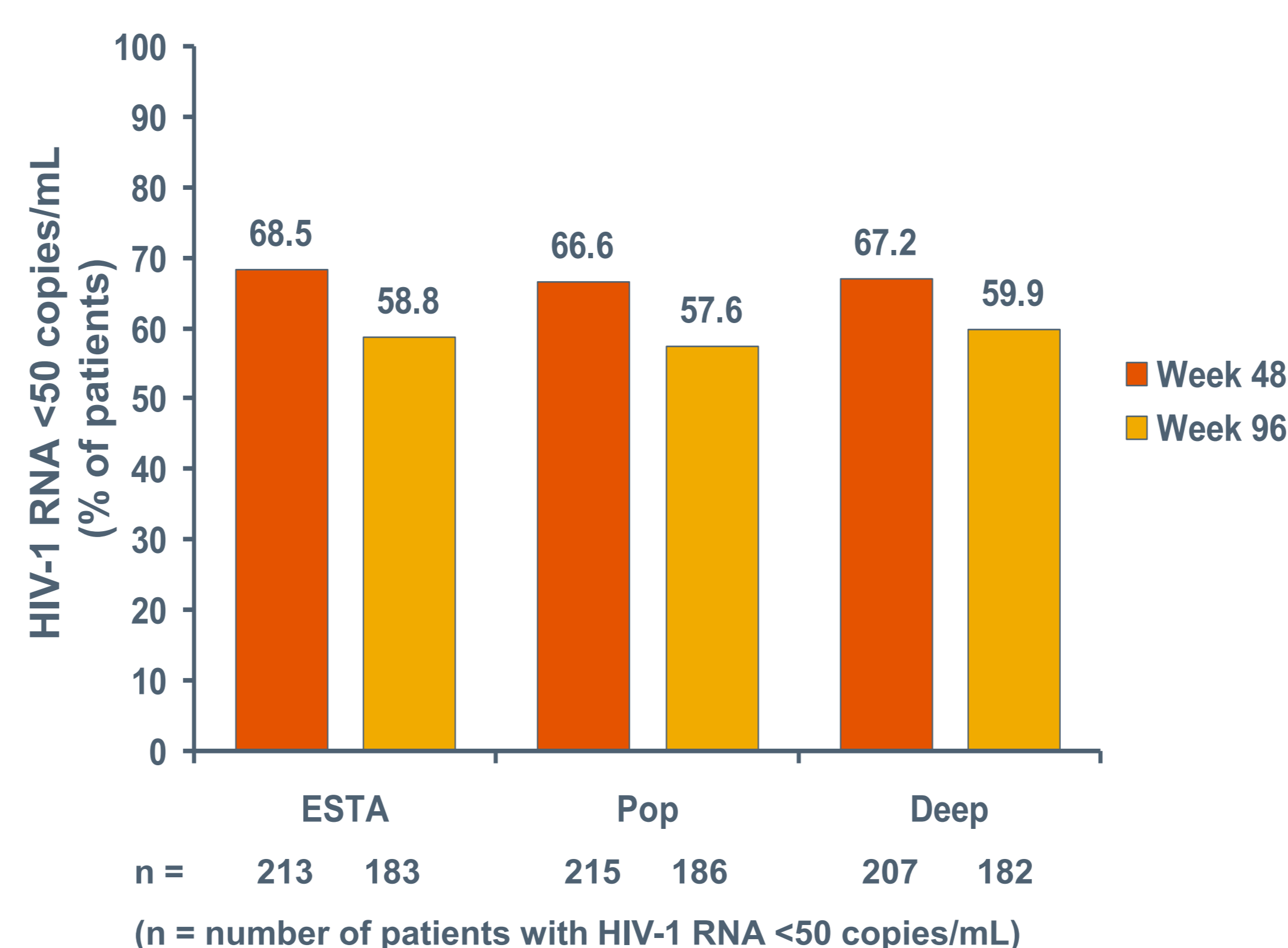


Figure 2. Subtype B Results : Equivalent Virological Responses at Weeks 48 and 96 in the Original Trofile Population Rescreened R5 With ESTA, Population Genotyping and 454 Genotyping

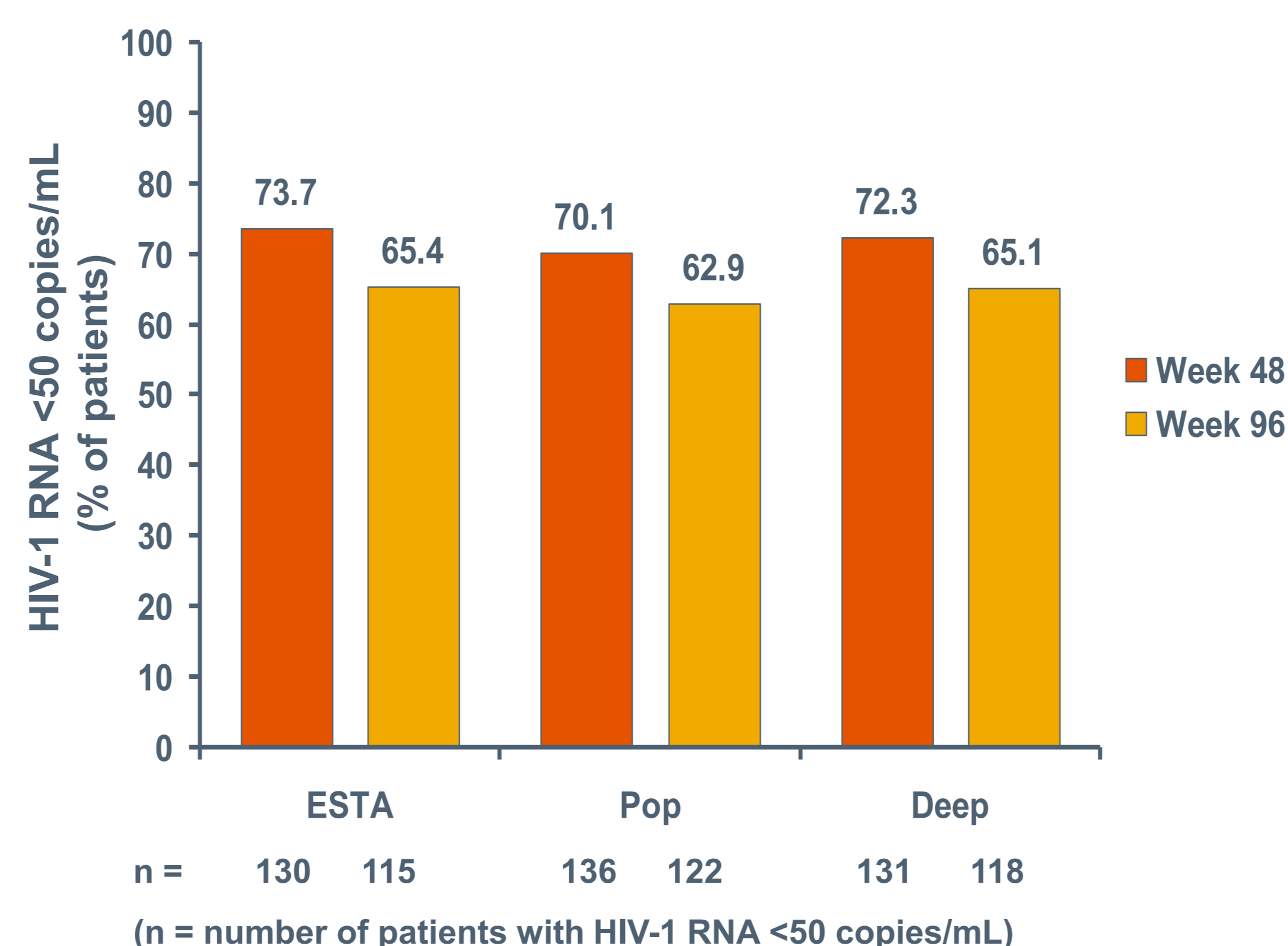


Figure 3. Subtype C Results: Equivalent Virological Responses at Weeks 48 and 96 in the Original Trofile Population Rescreened R5 With ESTA, Population Genotyping and 454 Genotyping

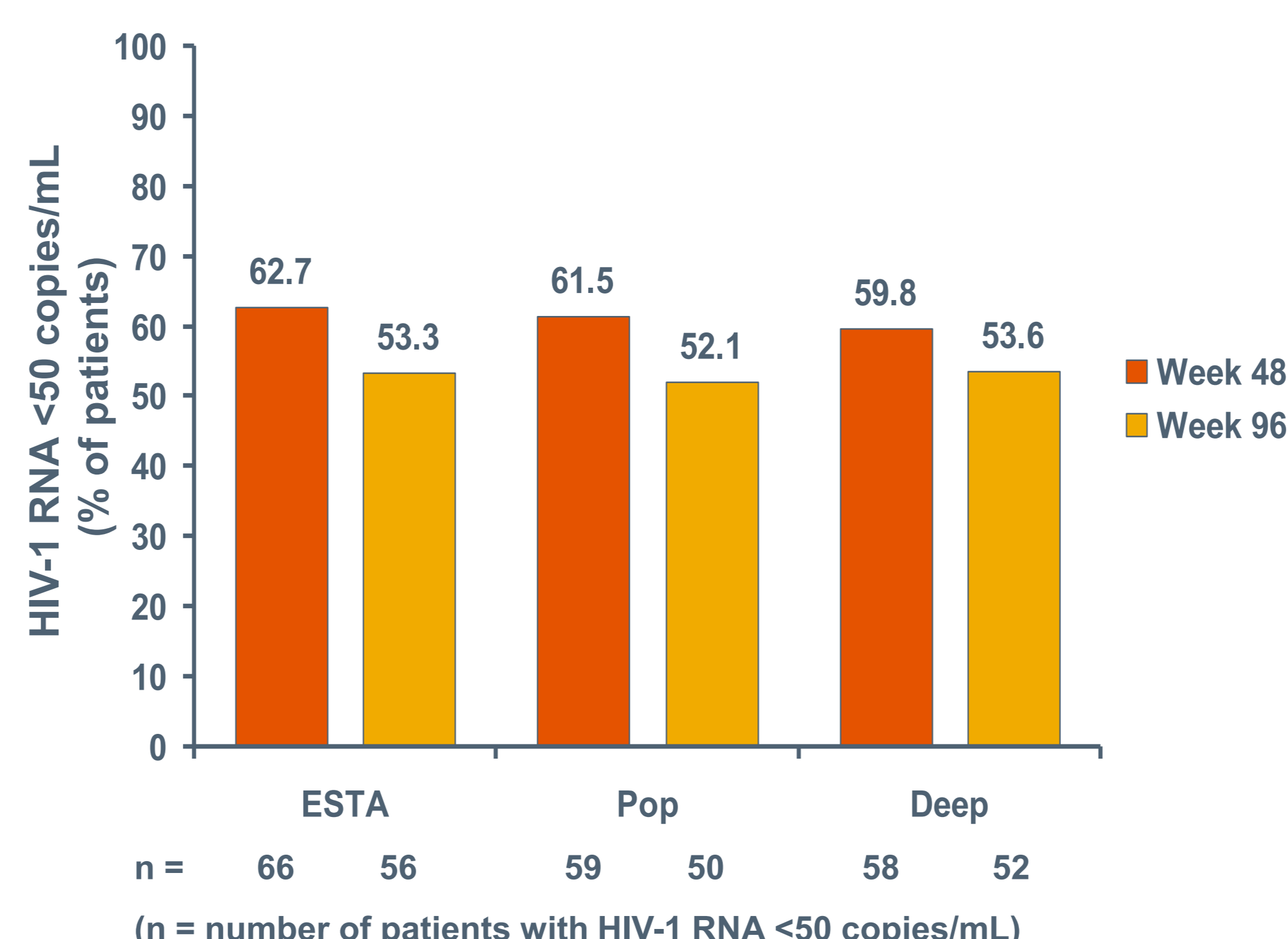


Figure 4. Results for Other Subtypes: Equivalent Virological Responses at Weeks 48 and 96 in the Original Trofile Population Rescreened R5 With ESTA, Population Genotyping and 454 Genotyping

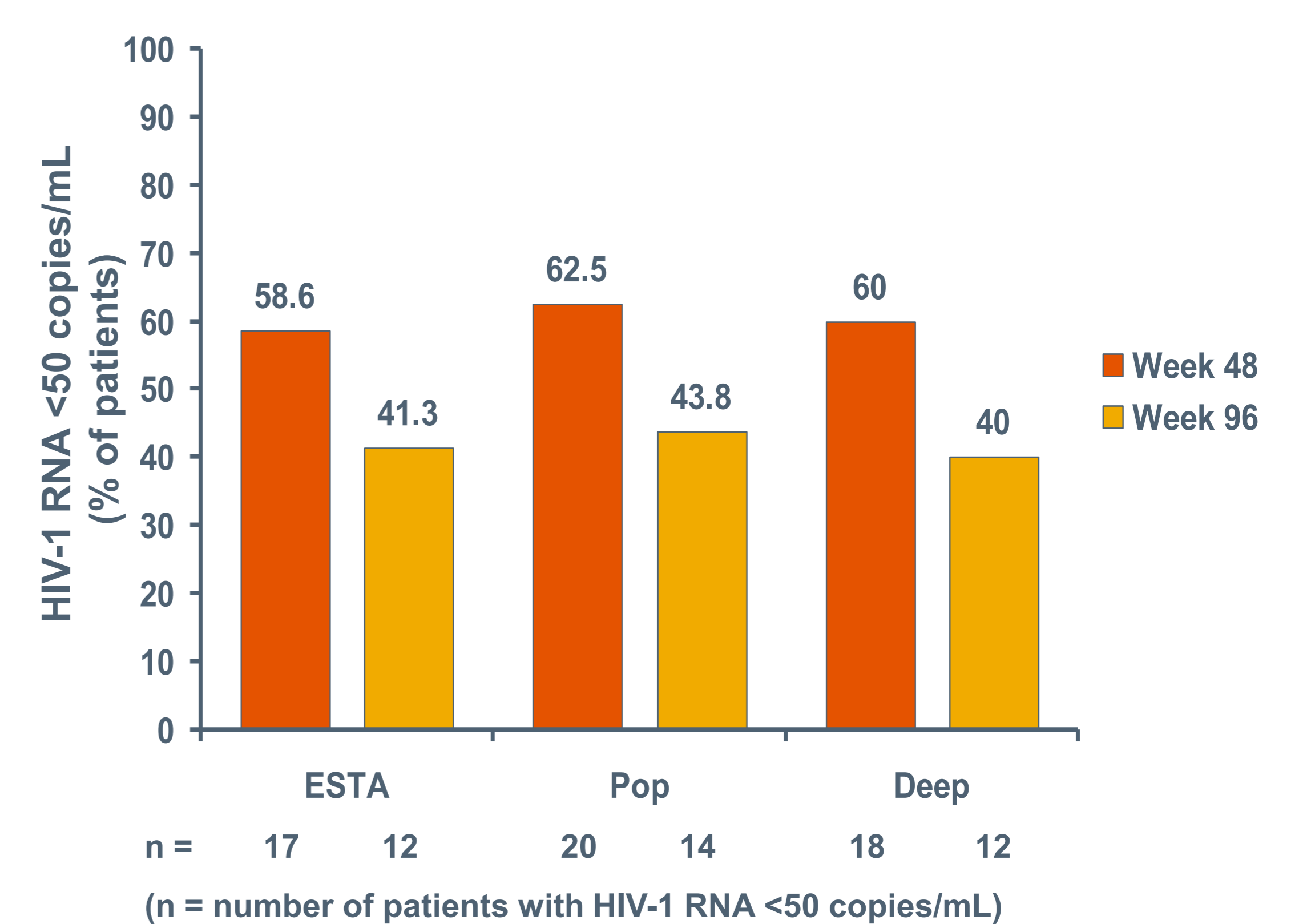


Table 3. Tropism Calls by ESTA, Population Sequencing and Deep Sequencing Show Similar “Sensitivity” and “Specificity” for Identifying Virologic Responders at 48 and 96 Weeks With Different HIV-1 Subtypes*

	Subtype B		Subtype C		Other Subtype	
	Sens (%)	Spec (%)	Sens (%)	Spec (%)	Sens (%)	Spec (%)
ESTA						
Week 48 responders	89.0	28.1	95.7	13.3	85.0	20.0
Week 96 responders	87.1	21.8	94.9	10.9	85.7	19.0
Population sequencing						
Week 48 responders	93.2	9.4	93.7	14.0	100	20.0
Week 96 responders	92.4	7.7	96.2	14.8	100	14.3
454 sequencing						
Week 48 responders	91.0	20.6	93.5	2.5	94.7	20.0
Week 96 responders	90.1	17.1	94.5	4.3	92.2	14.3

*In this analysis, sensitivity is defined as the proportion of responders (HIV-1 RNA < 50 copies/mL) who had R5 tropism calls at screening; specificity is defined as the proportion of non-responders who had non-R5 tropism calls at screening. These parameters are not true sensitivity and specificity but are included for illustrative purposes.

- There were no differences in the ability of the ESTA, population genotyping or 454 “deep” sequencing to predict virological responses across HIV-1 subtypes B, C and other subtypes
- The “sensitivity” of each test for predicting response was broadly similar across tests. The low “specificities” reflect the very small numbers of individuals with a non-R5 result, many of whom do subsequently respond to MVC.
 - These parameters are not true sensitivity and specificity but are included for illustrative purposes

Conclusion

- The ability of V3 population and deep sequencing genotyping methods to predict response to MVC was assessed in patients infected with different HIV-1 subtypes and found to be equivalent

References

- Cooper DA, et al. J Infect Dis. 2010; 201:803-13.
- McGovern R, et al. 17th Conference on Retroviruses and Opportunistic Infections. Presentation 92. 2010.
- Swenson L, et al. 17th Conference on Retroviruses and Opportunistic Infections. Presentation 545. 2010.
- Tressler R, 5th IAS Conference on HIV Pathogenesis, Treatment, and Prevention. Presentation TUPEA063. 2009.
- Eng S, et al. 5th IAS Conference on HIV Pathogenesis, Treatment, and Prevention. Presentation TUPEA062. 2009.