

# A Randomised, Prospective Study of real Phenotype (real-P) versus Virtual Phenotype® (virtual-P) Testing for Patients Failing Antiretroviral Therapy (ARVT): final analysis.

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## BACKGROUND

➤ Resistance testing is useful in the management of virological failure, as demonstrated in several retrospective and prospective studies using either genotypic or phenotypic assays.

➤ The best method to be used in clinical practice has not been determined.

➤ *Virtual phenotype* (a computerized system to interpret the genotype resistance testing) may have some advantages over phenotype, especially availability and cost. Furthermore, it overcomes one of the most important disadvantages of genotype: the interpretation of complex mutational patterns.

## OBJECTIVE

To determine the short-term virologic efficacy of changing antiretroviral therapy in failing patients based on *real-P* (Antivirogram™, Virco N.V.) compared to *virtual-P* (VircoGEN™II, Virco N.V.)

## METHODS

**Design:** Prospective, randomised, multicenter, double-blind study which compares the effect on virological response after changing antiretroviral therapy of the information provided by real-p vs virtual-p

**Setting:** five outpatient clinics of tertiary hospitals in Spain.

**Follow-up:** 24 weeks.

**Blind:** For attending clinicians the resistance assay performed at the laboratory was unknown. The format for the resistance testing was the same in both arms of the study. The drugs were categorised as S (sensitive), I (intermediate) and R (resistant) using former cut-off break points (<4 FR=S; 4-10 FR=I; >10 FR=R).

**Main variables:** Virological Response at 24 weeks. % of patients with < 400 copies/ml and Viral load decrease from baseline.

All patients were included in the analysis except those who died or were lost to follow-up before test information.  
• Patients with negative tests and those who were crossed over to the other arm to obtain a positive test were included in the analysis.  
• Missing data were considered failures  
• Treatment changes due to toxicity were not considered failures.

A censored analysis was performed excluding all negative test and crossover arm patients.

### Inclusion Criteria:

HIV Infection, > 18 years old, more than 3 months of ARVT, HIV-RNA > 3 log in 2 determinations. Patient and clinician agree to wait for resistance testing before prescribing the rescue regimen and written informed consent was signed.

### Exclusion Criteria:

Naive patients, adherence < 50%, if a period of ARVT interruption was planned, change of ARVT before resistance testing was available, wash-out of ARVT longer than 3 weeks, and viral load below the limit of detection before treatment interruption.

### Sample size and randomisation:

Three hundred patients, 150 to real-P (Antivirogram®) and 150 to virtual-P (virco-gen-II®), respectively were randomised, and stratified according to previous drug exposure (Stratum-1 < 2 drug classes and stratum-2 three drug classes), at a central level. An  $\alpha$  error and a  $\beta$  error of 0.05 and 0.10 were considered, respectively and a relevant difference of 20% between both methods and an estimation of losses of 10%, with a bilateral contrast.

### Laboratory procedures:

Genotype was performed in all patients that was possible, genes of retrotranscriptase and protease were extracted, amplified and sequenced (ABI PRISM™ 377). Sequences were aligned and compared with a wild strain in the Infectious Disease Laboratory at Hospital Ramon y Cajal. Patients were randomised to:

**Antivirogram®:** samples were sent to VIRCO lab where the recombinant phenotype assay was performed.

**VircoGEN II®:** text sequences were sent to Virco and interpreted with a relational database, where more than 20,000 patients genotype-phenotype were linked.

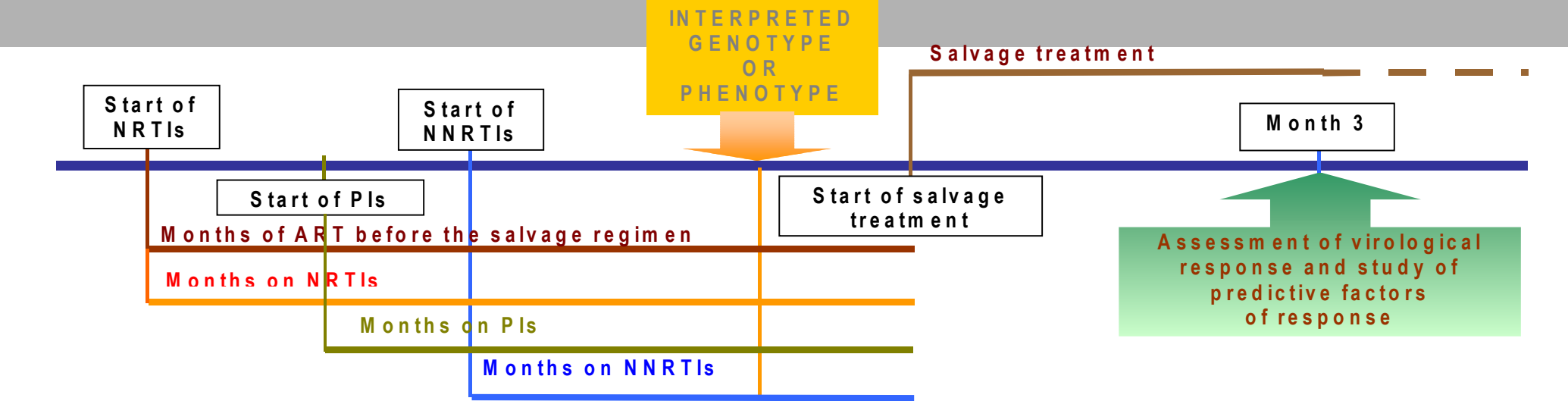
### Statistical analysis:

Imbalances between baseline characteristics by treatment allocation were assessed by significant tests.

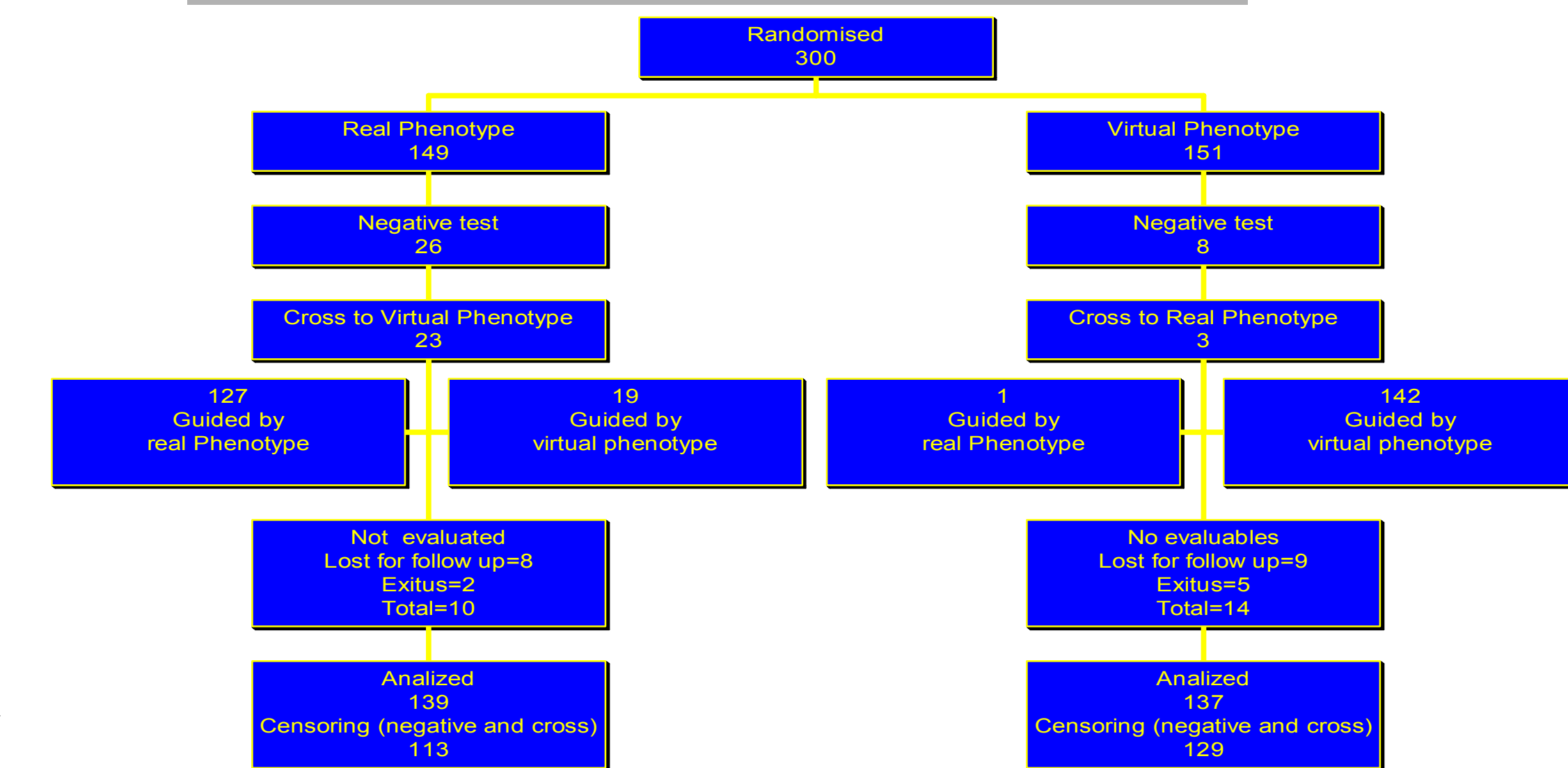
Univariate comparisons were performed with Chi-square (Fisher's exact where necessary) and independent-samples T test for categorical and continuous variables, respectively.

Multivariate analysis, logistic and lineal, using as dependent variables proportion of patients below the limit of detection and viral load decay were performed, adjusting for all potential confounding variables, baseline viral load, and adherence, at 6 month.

## Interventions



## Population Distribution



## RESULTS: Baseline Characteristics

	Real Phenotype 139	Virtual Phenotype 137
Sex (male) %	73	78
Age (years) mean ±SD	38 ±8	39 ±7
Mode of transmission %		
IVDA	55	52
Homosexual	24	27
Heterosexual	16	19
Other	5	2
AIDS (%)		
HIV-RNA (log) mean±SD	41	39
CD4 (cell/ml) mean ±SD	4 ±0.8	3.9 ±0.8
Strata (%)	355 ±250	336 ±207
1 (1 or 2 drug classes)	43	39
3 drug classes	57	61

	Real-Phenotype 139	Virtual -Phenotype 137	
Overall treatment length (months) mean±SD	65 ±32	64 ±30	NS
NRTIs (276)	65.2 ±31.9	62.4 ±30.2	
IP (248)	33.7 ±19.9	33.2 ± 15.9	
NNRTIS (87)	8.6 ±9.4	8.5 ±13.3	
Adherence with previous regimen (>90%)	71	74	
Pre-test ARTV washout (%)	4	4	
days ±SD	22.3±15.7	18±12.6	
Pos-test ARTV washout (%)	22	20	
days ±SD	127 ±108	105 ±89	
Delay from inclusion to new ARTV (days) mean±SD	87.5 ±58	87.2±58	
Attending Clinicians Test Fidelity (%)	89	87	

\*No significant imbalances were found between all the variables tested

## Multivariate linear regression analysis

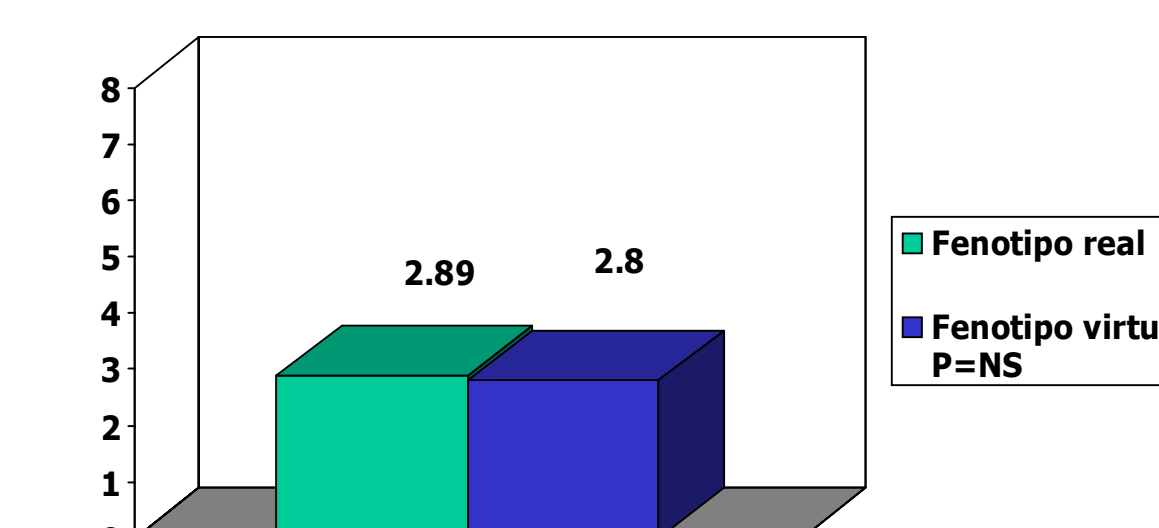
	$\beta$ coefficient	±SE	Significance
Adherence >90% (12-24s)	-1.335	±0.189	<0.001
Phenotype real vs virtual	0.305	±0.124	0.01
HIV-RNA baseline	0.507	±0.073	<0.001

In a multivariate linear regression analysis, after adjusting for all potential confounding variables (Baseline HIV\_RNA and adherence), a significant higher decrease was observed in the virtualP group [ $\beta$  Coef 0.3 ±0.124, p=0.01].

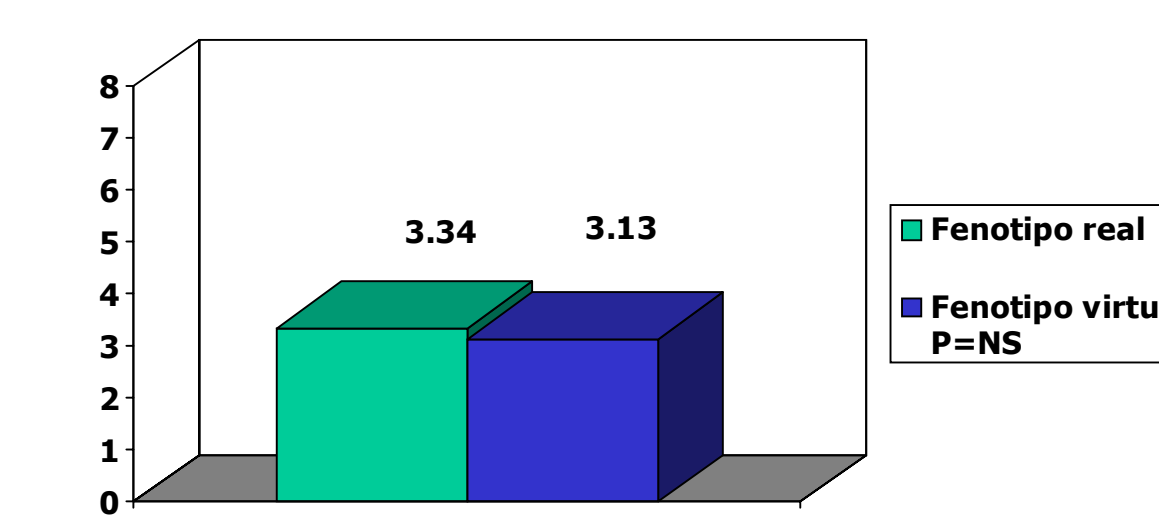
All those analysis were performed excluding patients with negative results or those who crossed to the other arm, and the results were similar to these (data not shown).

## Univariate analysis: Main outcome variables

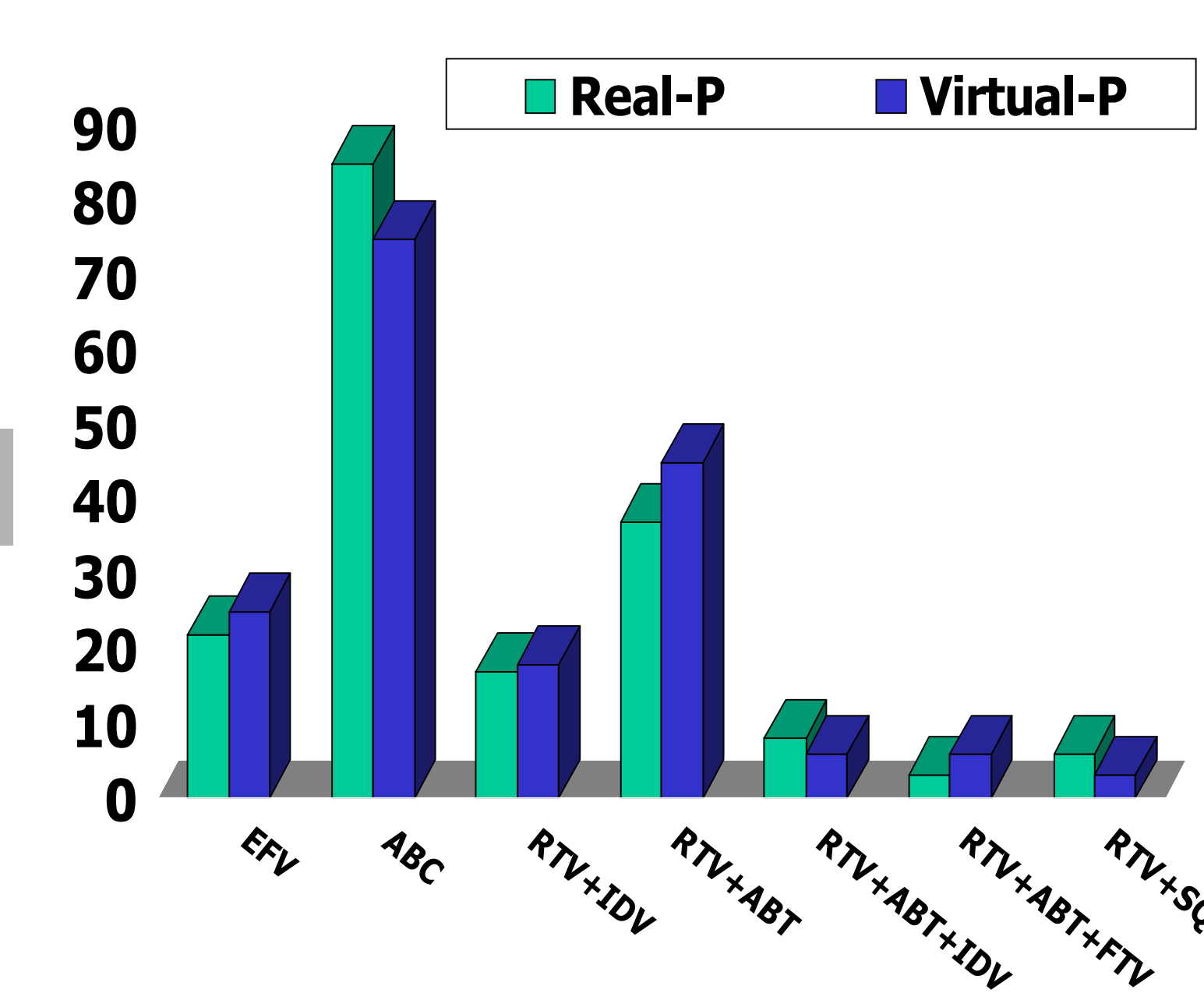
### Baseline Active drugs (Median)



### Potency-resistance score (Median)



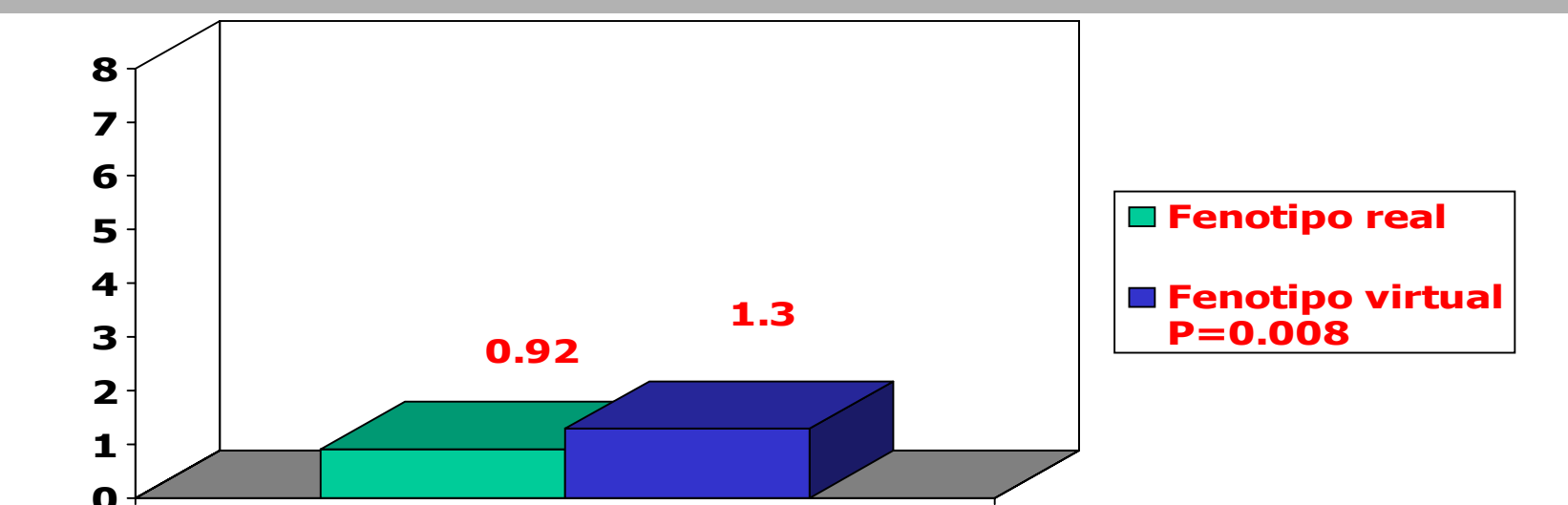
### Antiretroviral treatment prescribed



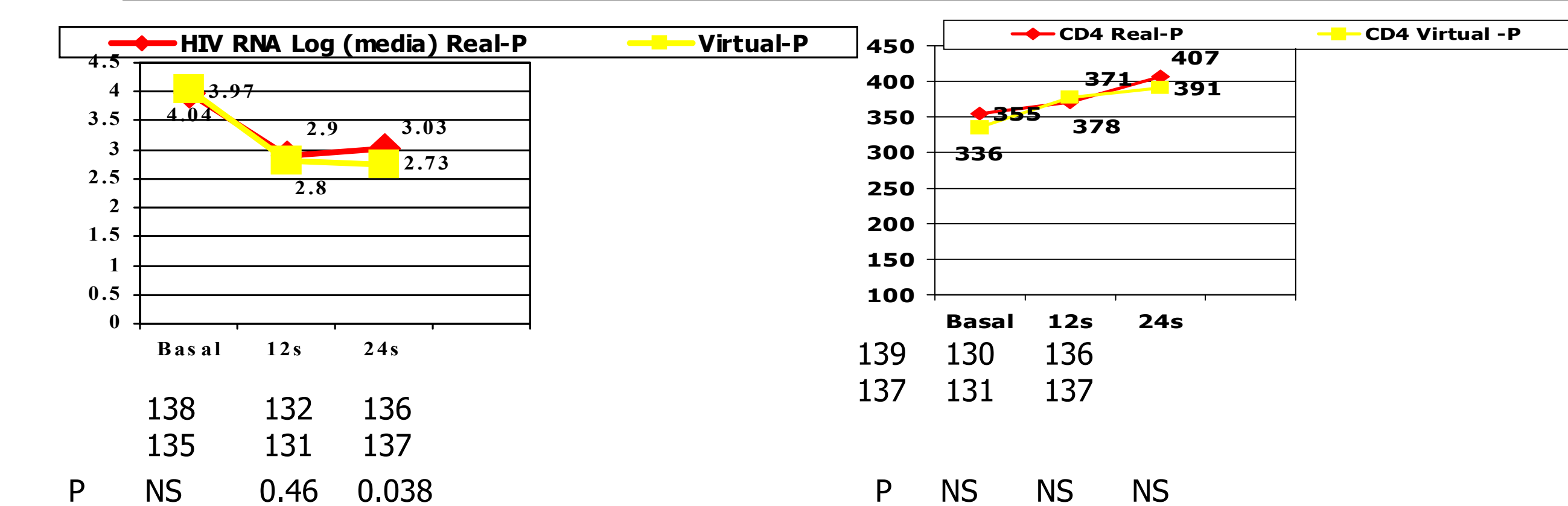
### Proportion of patients < 400 copies/ml, at 24 weeks

	Phenotype Real 139	Phenotype Virtual 137	P
Initial response (% HIV-RNA < 1 log o <2.5 at 12 W)	59.8	67.2	0.142
Initial adherence (>90%)	85.3	93.4	0.032
<b>Sustained response (% HIV-RNA &lt; 400c/ml at 24 S)</b>	<b>46.8</b>	<b>56.2</b>	<b>OR 1.47</b> <b>CI (2.38-0.9), 0.1</b>
Adherence 12-24 W (>90%)	86	89	0.49

### HIV-RNA decrease, at 24 weeks



### HIV-RNA and CD4 cell count evolution, at 24 weeks



## Conclusions

**Virtual phenotype performs as well as or better than real phenotype for guiding ARVT in subjects who have failed one or more antiretroviral regimens.**

**Virtual phenotype yield more positive results than Real phenotype**

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