

# IMMUNOLOGIC PREDICTORS OF DISCORDANCE BETWEEN CD4<sup>+</sup> T CELL AND HIV-1 RNA RESPONSES DURING ANTIRETROVIRAL THERAPY

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# INTRODUCTION

Human immunodeficiency virus type-1 (HIV-1) infection causes CD4+ T cell depletion and immunodeficiency. Effective antiretroviral therapy rapidly lowers plasma HIV-1 RNA concentrations, increases circulating CD4+ T cell numbers, and reduces morbidity and mortality. This CD4+ T cell response is biphasic. The initial rise reflects redistribution from tissues, and subsequent increases reflect overall increased CD4+ T cell numbers. The strength of the inverse correlation between HIV-1 RNA decline and CD4+ T cell increase is only moderate. Some patients with incomplete virologic suppression experience sustained CD4+ cell lymphocyte increases. Conversely, suppression of plasma viremia does not assure immune restoration. Such discordant responses suggest that factors, in addition to plasma HIV-1 RNA decline, influence CD4+ lymphocyte recovery. The present analysis examined whether selected immune markers could help predict discordance between virologic and immunologic responses.

# OBJECTIVES

- To determine whether levels of activation markers at baseline and on study drug therapy predict later discordance between HIV RNA and CD4 T cell responses.
- To determine whether functional markers and CD8 T cell counts at baseline and on study drug therapy predict later discordance between HIV RNA and CD4 T cell responses.
- To characterize the variability in CD4 T cell response to antiretroviral therapy despite comparable virologic responses.

# METHODS (1)

## Study Design and Subjects

- Subjects were identified retrospectively from 10 AACTG treatment protocols (ACTG 116, 117, 175, 229, 244, 246, 276, 315, 343, and 368).
- Protocols were diverse, ranging from treatment initiation in antiretroviral-naïve subjects, to changing therapy during incomplete virologic response, to discontinuing selected agents in a multidrug regimen.
- Subjects were evaluable for 16-week and/or 24-week evaluation intervals if both HIV-1 RNA and CD4<sup>+</sup> T cell data were available at the beginning and end of the interval.
- A greater than expected CD4<sup>+</sup> T cell increase for an HIV-1 RNA decline was I+/V- discordance ("good" immunologic and "poor" virologic). A less than expected CD4<sup>+</sup> T cell increase for an HIV-1 RNA decline was I-/V+ discordance. Other responses (I+/V+ and I-/V-) were concordant.
- Comparisons were between one discordant group (e.g. I+/V-) and the concordant group (I+/V+ plus I-/V-), while excluding the other discordant group (e.g. I-/V+).

# METHODS (2)

## Evaluation intervals

- Four evaluation intervals were examined:
  - Baseline to week 16
  - Baseline to week 24
  - Time of HIV RNA nadir to 16 weeks later
  - Time of HIV RNA nadir to 24 weeks later
- *HIV RNA nadir* was the time of first HIV RNA assay from which no subsequent result decreased by  $>0.3 \log_{10}$  copies/mL.

## Laboratory assays

- Activated lymphocytes were CD38 and HLA-DR positive as detected by flow cytometry.
- Naïve lymphocytes were CD45RA and CD62L positive as detected by flow cytometry.

# METHODS (3)

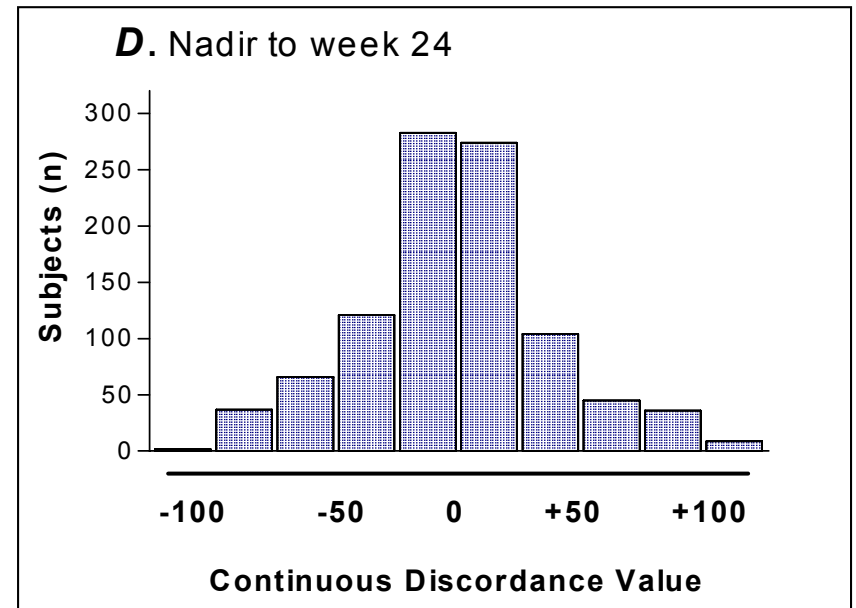
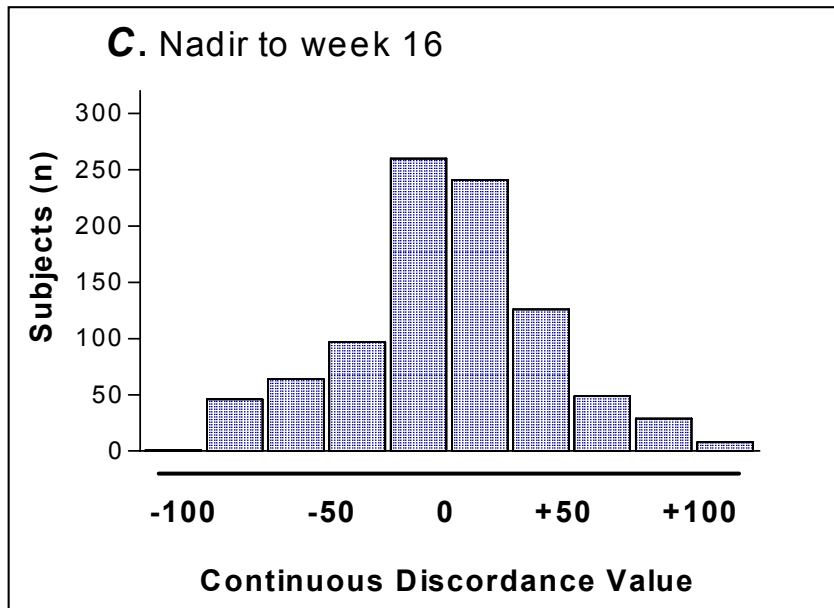
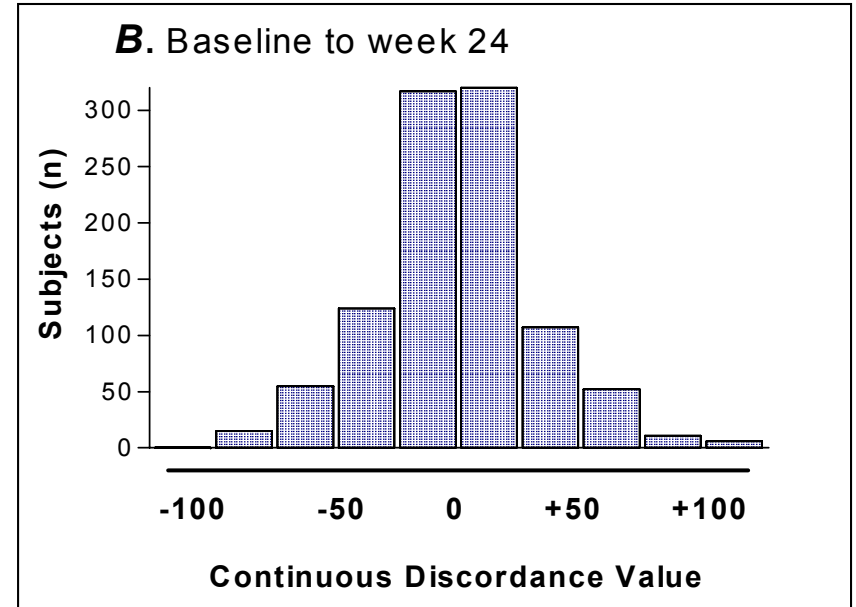
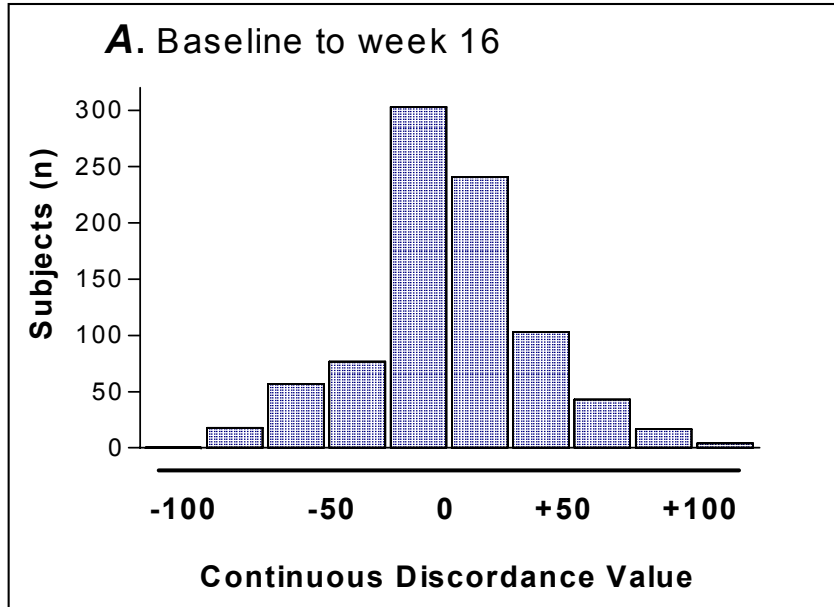
## Discordance Definitions

- Discordance was defined using 3 methods: *continuous*, *rank*, and *strict*.

### **Continuous definition of discordance**

- For each subject, absolute (or percentage) CD4+ T cell change over an interval was percentile-ranked relative to all evaluable subjects for that interval. A low rank indicated a more favorable response.
- For each subject, HIV-1 RNA response was percentile-ranked using a hierarchy of importance as follows: 1) HIV-1 RNA below lower limit of quantitation (LLQ) at interval end; 2) absolute  $\log_{10}$  HIV-1 RNA change; and 3) the greater of either LLQ or observed HIV-1 RNA at interval end. A low rank indicated a more favorable response.
- The continuous discordance value was HIV-1 RNA percentile-rank minus CD4+ T cell percentile-rank (Figure 1).

# Figure 1. Continuous Discordance Value Distributions



# METHODS (4)

## *Categorical definitions of discordance*

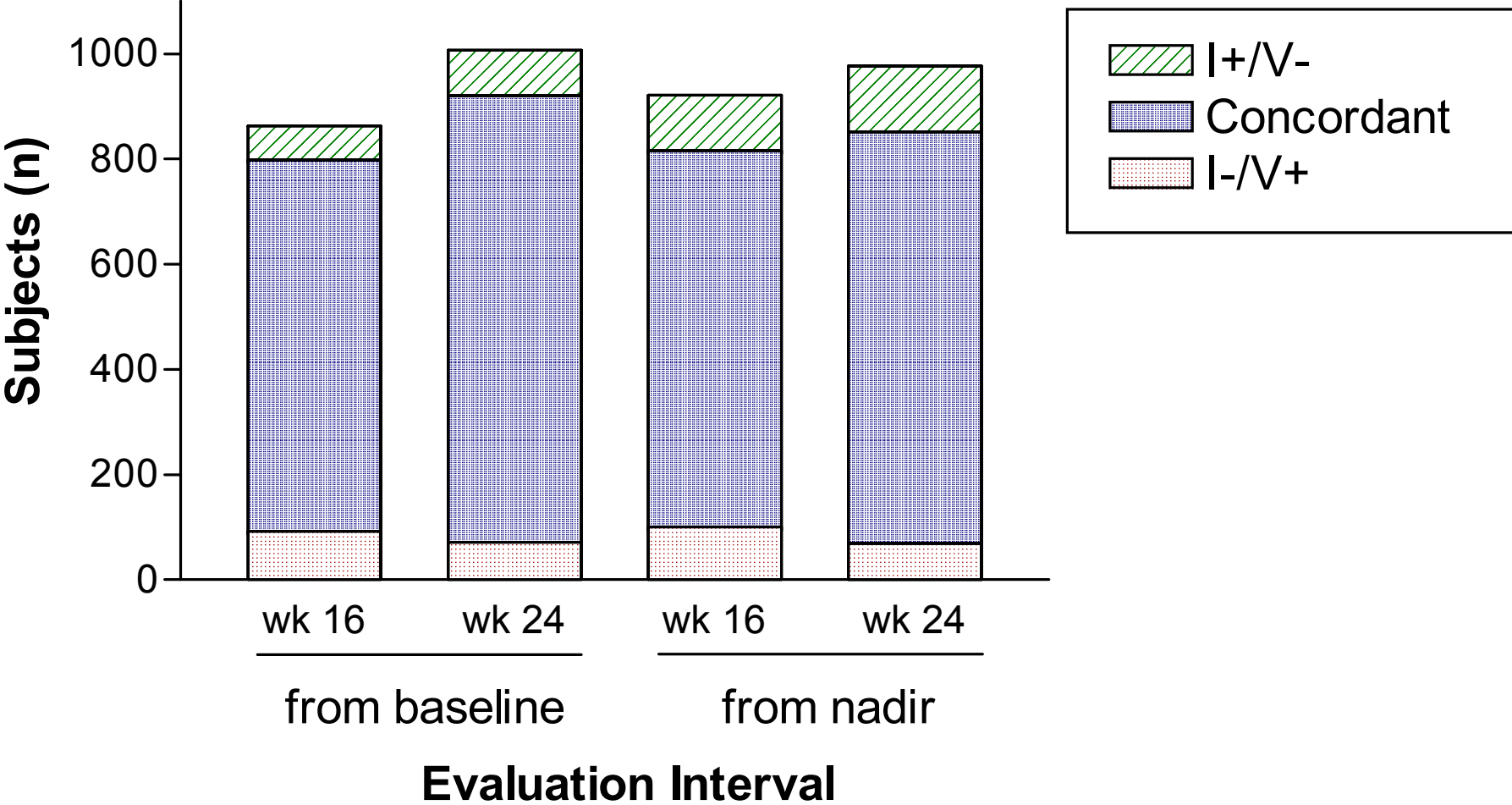
**Rank discordance:** I+/V- indicated HIV-1 RNA >LLQ at interval end AND change in absolute CD4+ T cell count in the top third for the group.

- I-/V+ discordance indicated HIV-1 RNA <LLQ at interval end AND change in absolute CD4+ T cell count in the bottom third for the group.
- Figure 2 shows distributions of rank discordance groups.

**Strict discordance:** Good (I+) and poor (I-) immunologic responses were CD4+ T cell increases greater or less than 100 cells/mm<sup>3</sup>, respectively.

- Good virologic responses (V+) were HIV-1 RNA values either <LLQ at interval end or decreases of  $\geq 1.0 \log_{10}$  copies/mL. A poor response (V-) was any lesser change.

# Figure 2. Rank Discordance Value Distributions



# METHODS (5)

## Recursive Partitioning Analysis

- For each interval, subjects were stratified on study, then randomly divided into *exploratory* and *confirmatory* data sets. Recursive partitioning was applied to the exploratory data set (for each interval, discordance definition, and adjustment method) to generate a null hypothesis that was tested in the confirmatory data set.

## Adjustment Procedures

- Two types of analyses were performed to adjust for age, race, gender, and PI use.
- ***Unadjusted:*** These variables were assessed together with immunologic and virologic variables for inclusion in a recursive partitioning tree.
- ***Adjusted:*** Forced to adjust for dichotomous variables: 1) young ( $\leq 50$  years of age) white male *versus* other, and 2) PI in the regimen *versus* other.
- For the continuous endpoint, adjustment involved subtracting stratum mean discordance value from each discordance value. For categorical analyses adjustment was done by analyzing each stratum separately.

# METHODS (6)

## Statistical Tests

- There were 76 primary hypothesis tests: all tests using continuous and rank categorical definitions based on absolute CD4+ T cell counts.
- Bonferroni adjustment for multiple testing with  $k=76$  for an overall type I error of 5% required  $p < 0.00066$  in the confirmatory data set.
- Of primary null hypotheses rejected, the overall type I error probability was  $< 5\%$ . Of primary null hypotheses rejected with  $p < .05$  based on the confirmatory data set (but with  $p \geq 0.00066$ ) the type I error for each hypothesis test is 5%, but the probability of at least one of these hypotheses being falsely rejected is  $> 5\%$ .
- Of each remaining non-primary null hypothesis rejected with  $p < .05$ , the evidence for the hypothesis is significant for exploratory purposes only.
- Each analysis was performed with and without forced adjusting for age, race, gender, and PI use.
- Analyses utilized two-sided Wilcoxon Rank Sum test using exact methods or Monte Carlo sampling, or two-sided Fisher's Exact test

# Example of I-/V+ Discordance Analysis Result: Baseline to Week 24 by Rank Analysis

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1. Criteria for discordant subset:

CD4		<b>OR</b>	<360	<b>OR</b>	360 - 455
CD4%	>37%		17% - 38%		27% - 38%
CD8			>914		>914

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2. **Discordant** **Concordant**

<b>In subset (46)</b>	<b>26</b>	<b>20</b>
<b>Not in subset (308)</b>	<b>44</b>	<b>264</b>

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3. Probability of I-/V+discordance:

In subset: 0.57 (0.41 - 0.71)  
 Not in subset: 0.14 (0.11 - 0.19)  
 (p = 0.0000001)

# RESULTS (1)

## Study subjects

- Characteristics of study subjects and clinical trials are shown in Table 1.
- Most study subjects were young white males, and most received a protease inhibitor in the study regimen.
- Evaluable subjects in each evaluation interval ranged from 863 (baseline to 16 weeks) to 1,007 (baseline to 24 weeks).
- Predictor variables with results available from at least 50 subjects per evaluation interval are listed in Table 2.

# TABLE 1. Subjects in Each Interval by Study Regimen

	Regimen	From baseline		From nadir	
		16 weeks	24 weeks	16 weeks	24 weeks
ACTG study					
116/117	ZDV or ddl	28	151	65	108
175	ZDV ± ddl ± ddC	29	48	45	43
229	ZDV ± ddC ± SQV	241	233	234	244
244	ZDV ± ddl ± NVP	16	14	15	17
246	ddl + d4T ± RTV	-	4	3	3
276	ZDV ± ddl	43	32	37	28
315	ZDV + 3TC + RTV	5	39	23	30
343	ZDV ± 3TC ± IDV	423	417	423	426
368	ABV + IDV + EFV	78	69	76	78
Female		16%	13%	16%	13%
PI use		77%	68%	77%	68%
Non-white		33%	31%	33%	31%
<i>Total</i>		863	1007	921	977

Abbreviations: 3TC = lamivudine, ABV = abacavir, d4T = stavudine, ddC = zalcitabine, ddl = didanosine, EFV = efavirenz, IDV = indinavir, NVP = nevirapine, PI = protease inhibitor, RTV = ritonavir, SQV = saquinavir, ZDV = zidovudine.

**TABLE 2. Subjects with Predictor Variable Data at Beginning of Evaluation Interval**

Predictor variable	From baseline		From nadir	
	16 weeks	24 weeks	16 weeks	24 weeks
HIV-1 RNA	863	1007	921	977
CD4+ T cells - <i>absolute, %</i>	863, 777	1007, 855	921, 856	977, 869
CD8+ T cells - <i>absolute, %</i>	831, 777	854, 854	855, 856	868, 869
Soluble neopterin	248	252	236	254
Soluble beta-2 microglobulin	247	256	235	254
CD4+/CD38+/DR+ T cells- <i>absolute, %</i>	204, 205	219, 219	214, 214	215, 215
CD8+/CD38+/DR+ T cells - <i>absolute, %</i>	203, 204	219, 219	213, 213	215, 215
CD4+/CD45RA+/62+ T cells - <i>absolute, %</i>	147, 148	164, 164	156, 156	157, 157
CD8+/CD45RA+/62+ T cells - <i>absolute, %</i>	146, 147	163, 163	157, 135	157, 135
CD19+ cells - <i>absolute, %</i>	66, 66	67, 67	63, 63	67, 67

# RESULTS (2)

## Factors distinguishing I+/V- discordance from concordance

- Predictors of I+/V- discordance are shown in Table 3.
- From baseline to weeks 16 and 24, only lower absolute and percent CD4<sup>+</sup> T cells predicted I+/V- discordance (corrected for multiple comparisons).
- In some analyses, lower activated CD4<sup>+</sup> T cell numbers and CD8<sup>+</sup> T cell numbers tended to predict I+/V- discordance at week 24.
- From nadir to weeks 16 and 24 thereafter, only lower absolute CD4<sup>+</sup> T cell counts and HIV-1 RNA concentrations at nadir predicted I+/V- discordance (corrected for multiple comparisons).
- One analysis from nadir to week 24 thereafter suggested that lower total and activated CD8<sup>+</sup> T cell percentages helped predict I+/V- discordance, but not when corrected for multiple comparisons.

# TABLE 3. Factors Predicting I+/V- Discordance<sup>a</sup>

Discordance definition	N <sup>b</sup>	Adjusted	CD4 count (cells/mm <sup>3</sup> )	CD4 (%)	HIV RNA (log10 c/mL)	Other	P value
<b>Baseline forward</b>							
<i>Week 16</i>							
Continuous	211	No	<285	-	-	-	0.0000014
Continuous	213	Yes	<286	-	-	-	0.0000426
Continuous <sup>c</sup>	104	No	-	<19%	-	-	0.0000479
<i>Week 24</i>							
Continuous	208	No	<217	-	-	-	<.0000001
Continuous	208	Yes	<217	-	-	-	<.0000001
Continuous <sup>c</sup>	11	No	-	-	-	Activated CD4 <15 cells/mm <sup>3</sup>	0.00878
Continuous <sup>c</sup>	11	Yes	-	-	-	Activated CD4 <15 cells/mm <sup>3</sup>	0.00503
Rank <sup>c</sup>	19	Yes <sup>d,e</sup>	-	-	-	CD8 <464 cells/mm <sup>3</sup>	0.00445
<b>Nadir forward</b>							
<i>Week 16</i>							
Continuous	198	No	<272	-	-	-	<.0000001
Continuous	198	Yes	<272	-	-	-	0.0000016
<i>Week 24</i>							
Continuous	137	No	<309	-	<4.15	-	0.000002
Continuous	108	Yes	<309	-	<3.65	-	0.000036
Rank	9	No	-	-	2.70 to 3.85	Naïve CD4 >35%, Age 31 to 39	
			180 to 324	-	2.35 to 3.85	Naïve CD4 <35%, CD8 <61%,	0.0228

<sup>a</sup> Subset at risk for discordance satisfies all criteria in every column; <sup>b</sup>N, subjects meeting subset definition in the confirmatory dataset; <sup>c</sup>Change in CD4+ T cell *percentage* was percentile ranked; <sup>d</sup>Analysis includes only white males 50 or fewer years old; <sup>e</sup>Regimen includes a PI.

# RESULTS (3)

## Factors distinguishing I-/V+ discordance from concordance

- Predictors of I-/V+ discordance are shown in Tables 4 and 5.
- From baseline to weeks 16 and 24, I-/V+ discordance was most consistently predicted by higher absolute and percent CD4+ T cells and lower HIV-1 RNA levels (corrected for multiple comparisons).
- In one analysis, lower naïve CD4+ T cells was predictive.
- Among 35 subjects with HIV-1 RNA <4.53 copies/mL, absolute naïve CD4+ T cell counts <179 cells/mm<sup>3</sup>, and receiving a PI (meeting all 3 criteria), the probability of I-/V+ discordance was 0.71. The probability among 224 subjects not in this subset was 0.19.
- Higher absolute CD8+ T cell counts tended to predict discordance in some analyses.
- From HIV-1 RNA nadir to weeks 16 and 24 thereafter, I-/V+ discordance was most consistently predicted by higher CD4+ and CD8+ T cell counts and by HIV-1 RNA levels <LLQ.

# TABLE 4. Baseline Factors Predicting I-/V+ Discordance<sup>a</sup>

Discordance definition	N <sup>b</sup>	Adjusted	CD4 count (cells/mm <sup>3</sup> )	CD4 (%)	HIV RNA (log <sub>10</sub> c/mL)		Other		P value
<i>Week 16</i>									
Rank <sup>c</sup>	17	No	591 to 809	<42%	<4.02	-	Age <56	-	
			-	-	<4.35	-	Age >55	-	0.0247
Continuous	213	No	>284	-	-	-	-	-	0.0000014
Continuous	211	Yes	>285	-	-	-	-	-	0.0000427
Strict	35	No	-	-	<4.53		Naïve CD4 <179 /mm <sup>3</sup>	PI use	<.0000001
Strict	47	Yes <sup>e,f</sup>	-	--	<3.79	-	-	-	0.0289
Strict	78	Yes <sup>e,g</sup>	-	-	<4.28	-	-	-	0.00547
Rank <sup>c,h</sup>	41	No	-	30 to 41	<3.93	-	-	-	0.0345
			-	>40%	-	-	-	-	
Rank <sup>h</sup>	15	Yes <sup>e,f</sup>	-	>35	-	-	-	-	0.00152
Continuous <sup>h</sup>	120	Yes	-	>18	-	-	-	-	0.000203
<i>Week 24</i>									
Continuous	287	No	>216	-	-	-	-	-	<.0000001
Continuous	287	Yes	>216	-	-	-	-	-	<.0000001
Strict <sup>c</sup>	23	No	-	-	<4.01	CD8 >1211 /mm <sup>3</sup>	Naïve CD4 >163 /mm <sup>3</sup>	-	
			-	-	<4.55	CD8 >52%	Naïve CD4 <164 /mm <sup>3</sup>	PI use	0.0178
Rank <sup>c,h</sup>	46	No	-	>37	-	-	-	-	<.0000001
			<360	17 to 38	-	CD8 >914 /mm <sup>3</sup>	-	-	
			360 to 455	27 to 38	-	CD8 >914 /mm <sup>3</sup>	-	-	
Rank <sup>h</sup>	18	Yes <sup>e,f</sup>	-	>34	-	-	-	-	0.00002
Rank <sup>c,h</sup>	38	Yes <sup>f,g</sup>	>332	>36	-	-	-	-	0.0298
			<332	>18	-	-	-	-	
Continuous <sup>h</sup>	120	No	-	>18	-	-	-	-	0.0000481
Continuous <sup>h</sup>	120	Yes	-	>18	-	-	-	-	0.0002029

<sup>a</sup> The subset at risk for discordance satisfies all criteria in every column; <sup>b</sup>N, subjects meeting subset definition in the confirmatory dataset; <sup>c</sup>For results with >1 row, the group at risk for discordance is comprised of subgroups represented by a separate rows; <sup>e</sup>Includes only white males 50 or fewer years old; <sup>f</sup>Regimen includes a PI; <sup>g</sup>Change in CD4+ T cell *percentage* was percentile ranked.; <sup>h</sup>Analysis includes only subjects other than young white males.

# TABLE 5. Nadir Factors Predicting I-/V+ Discordance<sup>a</sup>

Discordance definition	N <sup>b</sup>	Adjusted	CD4 count (cells/mm <sup>3</sup> )	CD4 (%)	HIV RNA (log <sub>10</sub> c/mL)		Other		P value
<i>Week 16</i>									
Rank	13	Yes <sup>g,f</sup>	>700	<36	-	-	-	-	0.00617
Continuous	263	No	>271	-	-	-	-	-	<.0000001
Continuous	161	Yes	>271	-	-	CD8 >800 /mm <sup>3</sup>	-	-	0.000004
Strict	237	No	-	-	<2.70	-	-	-	<.0000001
Strict	11	Yes <sup>e,l</sup>	-	-	<2.70	-	-	-	0.000088
Strict	103	Yes <sup>e,f</sup>	-	-	<2.70	-	-	-	<.0000001
Strict	103	Yes <sup>f,g</sup>	-	-	<2.55	-	-	-	<.0000001
<i>Week 24</i>									
Rank <sup>c</sup>	27	No	>570	-	-	CD8 >710 /mm <sup>3</sup>	Age <31	-	0.01044
			>570	-	-	CD8 >710 /mm <sup>3</sup>	Age >44	-	
Continuous	241	No	>308	-	-	-	-	-	0.00432
Continuous	241	Yes	>308	-	-	-	-	-	0.00169
Strict <sup>c</sup>	102	No	>421	-	<2.70	CD8 >916 /mm <sup>3</sup>	White race	-	<.0000001
			>421	-	<2.70	CD8 >1372 /mm <sup>3</sup>	Non white race	-	
			283 to 421	-	<2.70	CD8 >530 /mm <sup>3</sup>	-	-	
Strict	12	Yes <sup>e,f</sup>	-	-	<2.70	CD8 >1330 /mm <sup>3</sup>	-	-	0.00806
Strict <sup>c</sup>	64	Yes <sup>f,g</sup>	>312	-	<2.70	CD8 >889 /mm <sup>3</sup>	-	-	0.000161
			312 to 423	-	<2.70	CD8 <890 /mm <sup>3</sup>	-	-	
Rank <sup>h</sup>	3	No	-	>38	-	Age >46	-	-	0.03
			283 to 421	-	<2.70	CD8 >530 /mm <sup>3</sup>	-	-	
Strict	12	Yes <sup>e,f</sup>	-	-	<2.70	CD8 >1330 /mm <sup>3</sup>	-	-	0.00806
Strict <sup>h</sup>	64	Yes <sup>f,g</sup>	>312	-	<2.70	CD8 >889 /mm <sup>3</sup>	-	-	0.000161

<sup>a</sup> The subset at risk for discordance satisfies all criteria in every column; <sup>b</sup>N, subjects meeting subset definition in the confirmatory dataset; <sup>c</sup>For results with >1 row, the group at risk for discordance is comprised of subgroups represented by a separate rows; <sup>e</sup>Includes only white males 50 or fewer years old; <sup>f</sup>Regimen includes a PI; <sup>g</sup>Change in CD4+ T cell *percentage* was percentile ranked.; <sup>h</sup>Analysis includes only subjects other than young white males.

# CONCLUSIONS

- The most consistent predictors of subsequent discordance were CD4+ T cell counts and percentages, and HIV-1 concentrations.
- In some analyses, absolute CD8+ T cell counts, CD8+ T cell percentages, and absolute naïve CD4+ T cell counts also identified subjects most likely to experience subsequent discordance.
- Soluble neopterin and  $\beta$ -2 microglobulin did not predict discordance.
- Absolute and percent CD19+ cells was not predictive, although few subjects were evaluable for this marker.
- Lower numbers of naïve CD4+ T cells helped to predict I-/V+ discordance.
- The predictive value of absolute and percentage CD8+ T cells for I-/V+ discordance may reflect better control of HIV-1 replication.
- Recursive partitioning allows relevant breakpoints to be empirically identified without defining breakpoints a priori, minimizing investigator bias.
- This approach may ultimately identify subgroups of patients at risk for discordant responses in ways most applicable to clinical practice.