

Abstract 499M

Immune Correlates of a Discordant CD4⁺/VL Response to PI-Based HAART. S. Sufka*, G. Ferrari, G. Sempowski, H. Staats, V. Gryszowka, V. Teaberry, K. Weinhold, C. Hicks, Duke Univ Med Ctr, Durham, NC.

Background: A discordant response to highly active antiretroviral therapy (HAART) occurs when CD4⁺ T cell counts are stable or increased over time despite detectable viral load (VL). The role of humoral and cellular immune responses in this phenomenon are still undetermined.

Methods: 30 HIV⁺ patients (22 male, 8 female; 17 W, 13 AA) on protease inhibitor (PI)-based HAART were studied in 3 groups: Failure (F): VL increasing, CD4⁺ decreasing; Discordant (D): VL 500-5000 copies/mL, CD4⁺ > 200/mm³ with increasing trend sustained > 2 years; Success (S): VL < 50 copies/mL, CD4⁺ > 200/mm³ with increasing trend sustained > 2 years. All subjects were treatment adherent as judged by history and resistance profile. Measures of immune function included: CD8⁺ response to gag, rev, tat, vpr antigens by Interferon- γ ELISpot; quantitation of T-cell subsets and CD38 antibodies bound per cell (ABC) by flow cytometry; CD4⁺ and CD8⁺ sjTRECs; serum IgG responses to gp120, gp41 peptide, MN V3 peptide. Comparisons between groups were made by Wilcoxon Rank Sum, Kruskal-Wallis, or Fisher's Exact Test.

Results: Groups did not differ significantly in age, gender, IgG responses, or CD4⁺/CD8⁺ sjTRECs, although sjTRECs trended lower in D vs. S or F. Cellular activation as measured by CD38⁺HLA-DR⁺ expression trended greater for F vs. D on CD4⁺ and CD8⁺ cells (median CD4⁺: F=15% D=11% p=0.06; CD8⁺: F=37% D=31% p=0.08). Median CD38 ABC was significantly greater for all T-cell subsets for F vs. D (naïve CD4⁺: F=15323 D=8911 p=0.01; memory CD4⁺: F=12319 D=6333 p=0.005; activated CD4⁺: F=17297 D=11119 p=0.04; activated CD8⁺: F=20357 D=8413 p=0.008). CD8⁺ responses to gag, tat were greater in D vs. S or F. (Median spot forming cells/10⁶ cells: gag D=1113 S=434 F=760; D vs. S:p=0.02, D vs. F:p=0.2; tat D=65 S=16 F=41; D vs. S:p=0.06, D vs. F:p=0.6) The ELISpot assay showed a detectable CD4⁺ response to gag in 5/9 D subjects, 3/8 F, 0/10 S.

Conclusions: Patients with a discordant CD4⁺/VL response to PI-based HAART have decreased immune activation and enhanced HIV-directed CD4⁺ T-helper and CD8⁺ responses that may contribute to control of HIV replication and immune homeostasis.

Background

HAART regimens using 2 NRTIs and a PI have been shown to ↓VL, ↑CD4⁺ cells and slow progression to AIDS^{1,2}. Current treatment guidelines³ recommend suppression of VL to the lowest possible level, ideally below the level of detection of the assay. However, a large number of patients are unable to achieve or maintain full viral suppression⁴⁻⁶.

Patients with discordant CD4⁺/VL responses are able to maintain or increase their CD4⁺ cell counts in the face of persistent, detectable viremia. Discordant patients have a reduced risk of AIDS complications, prolonged survival, and immunologic benefit⁷⁻¹⁰. However, mechanisms which allow control of viremia and maintenance of CD4⁺ counts have not been elucidated.

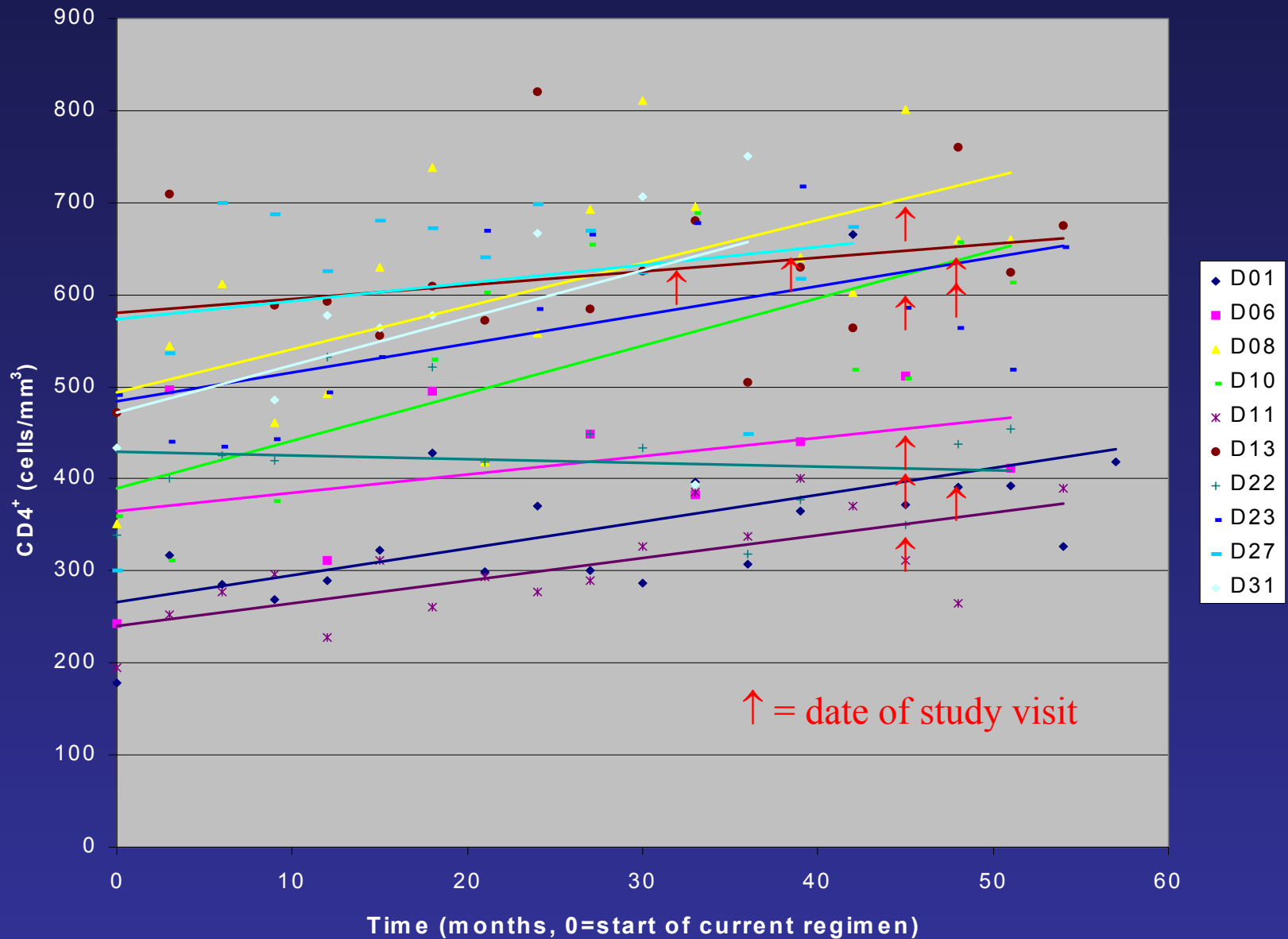
Study Objectives

- Determine the role of host, viral, immune, and treatment factors in patients experiencing full viral suppression, a discordant response, or treatment failure
- Identify potential mechanisms by which patients with a discordant response maintain their CD4⁺ count in the face of persistent, low-level viremia

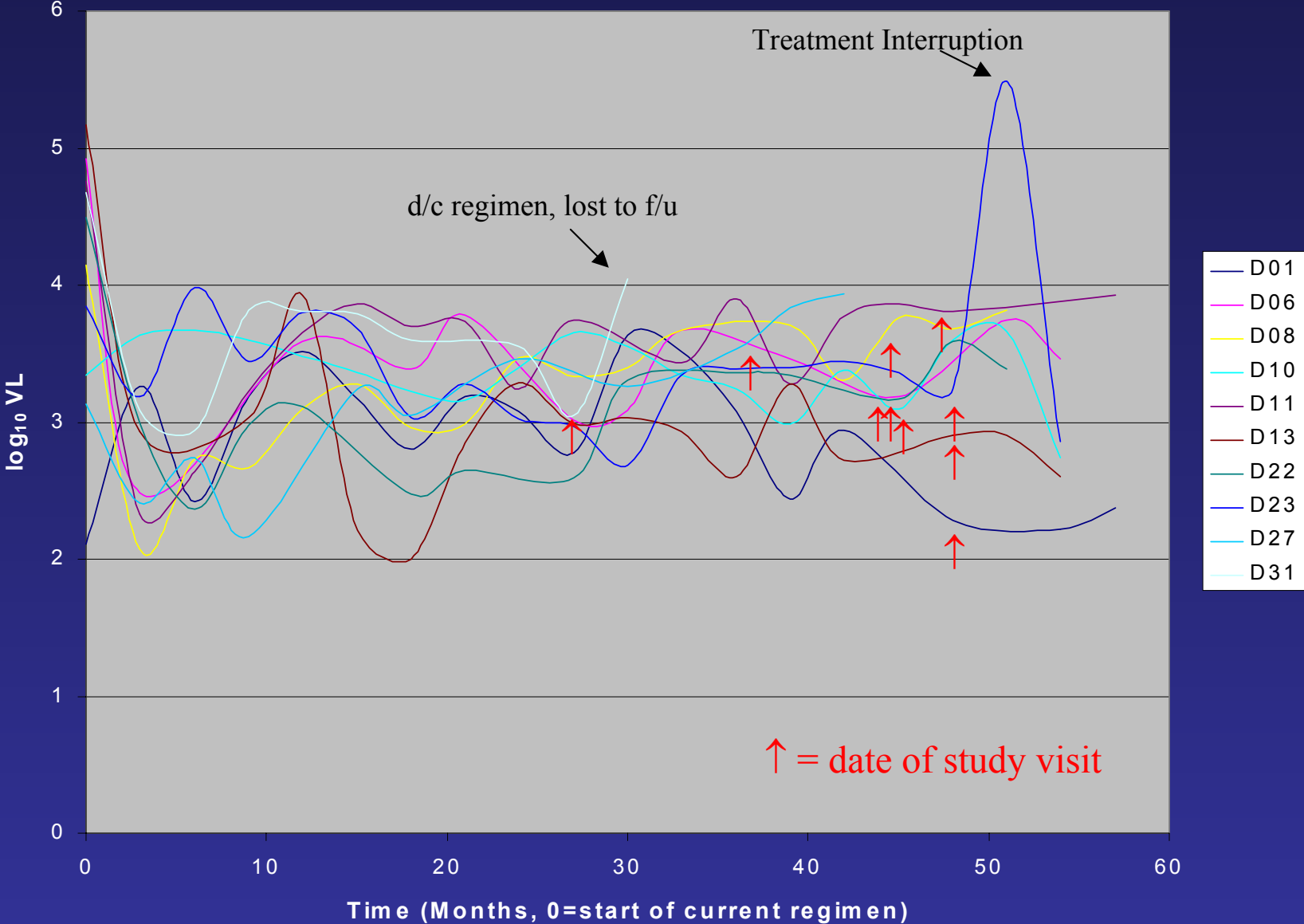
Study subjects

- 30 HIV+ patients on stable PI-based HAART
 - all treatment adherent by history and resistance profile
- Discordant group (n=10):
 - VL 500-5000 copies/mL; CD4⁺ > 200/mm³ with stable or ↑ trend sustained > 2 years
- Success group (n=10):
 - VL < 50 copies/mL; CD4⁺ > 200/mm³ with stable or ↑ trend sustained > 2 years
- Failure group (n=10):
 - VL increasing; CD4⁺ decreasing after initial response

Group D: CD4⁺ Cell Counts Over Time



Group D: VL Over Time



Methods

This was a single-center, cross-sectional study. Subjects were identified by retrospective chart review of CD4⁺ and VL data. Inclusion criteria: (1) > 18 years of age; (2) confirmed diagnosis of HIV infection; (3) current HAART regimen includes a protease inhibitor; (4) documented off-therapy viral load >50,000 copies/mL and/or CD4⁺ nadir <50 cells/mm³; (5) meets criteria for one of the three study groups. Exclusion criteria: (1) therapy was initiated during primary infection; (2) treatment regimen included IL-2 or hydroxyurea; (3) prior or current therapy with immuno-modulatory drugs; (4) non-adherence to HAART regimen by history or complete absence of genotypic resistance; (5) history of vaccination or febrile illness in the past 4 weeks.

Immune Factors Assessed

- CD8⁺ response to gag, rev, tat, vpr antigens by Interferon- γ ELISpot
- Quantitation of T-cell subsets and CD38 antibodies bound per cell (ABC) by flow cytometry
- CD4⁺ and CD8⁺ sjTRECs
- Serum IgG responses to gp120, gp41 peptide and MN V3 peptide by ELISA

Statistical Analysis

All comparisons among groups were made using non-parametric tests (Wilcoxon Rank Sum, Kruskal-Wallis, or Fisher's Exact Test). Values are reported as median (IQR). All p-values are 2-sided. All statistical analyses were performed with the use of SAS version 8.2 (SAS Institute, Cary, NC).

IFN- γ ELISpot

Interferon-gamma (IFN- γ) ELISpot assay was performed using 4 pools of 122, 23, 27, and 22 15-mer peptides overlapping by 11 amino acids representing the Gag, Tat, Rev, and Vpr gene products, respectively. Peptides were synthesized based on the sequence of the HXB2 (Gag) and Consensus B (Tat, Rev, and Vpr) isolates. Peptides were used at a final concentration of 1 μ g/ml. Cells were thawed and rested overnight at 2×10^6 cells/ml in RPMI 1640 containing 10% FCS serum before stimulation with the peptide pools for subsequent 16-18 hours. Anti-CD8 mAb-coated magnetic microspheres (Dynal, Oslo) were used to selectively deplete effector cell population. Goat anti-mouse IgG-coated beads were used as control. Cell populations were plated at $1-2 \times 10^5$ cells/well. Cells alone and PHA stimulated cells were used as negative (background) and positive controls, respectively. Based on the results obtained with PBMC from 7 seronegative individuals, results were considered positive if the number of spot per million cells (SFC/ 10^6 cells) in the experimental wells was three fold higher than the background and >20 SFC/ 10^6 cells. Responses were considered mediated by CD8⁺ cells if the number of spots in the CD8-depleted population was less than 50% of the spot in the undepleted population. Spots were counted by an independent source with a CTL Imager reader.

Flow Cytometry

Fresh EDTA anti-coagulated whole blood was processed within 6 hours of draw. Monoclonal antibodies from Becton Dickinson (San Jose, CA) were added in the following 4-color PE, FITC, PerCP, and APC combinations: MsIgG/ MsIgG/ CD4/ MsIgG, CD38/ CD45RA/ CD4/ CD62L, and CD38/ HLA-DR/ CD8/ CD4. In addition, pre-mixed Multitest™ CD3/ CD8/ CD45/ CD4 was added. Cells were treated with FACS™ Lysing Solution (BDIS), washed with PBS, fixed in PBS-1% formaldehyde, and acquired immediately on a FACSCalibur™ cytometer. CaliBRITE™3, CaliBRITE™ APC, and QuantiBRITE™ were used to validate instrument performance and standardize fluorescence intensity. Quantitative immunofluorescence values in units of Antibody Binding Capacity (ABC) were generated by relating PE median fluorescence intensity values, which were obtained from cells of interest, to the quantitative standard QuantiBrite™. To minimize assay variability, identical reagent lots were used for sample preparation and instrument set-up. Flow cytometric acquisition and analyses were performed using CellQuest™ software (BD, San Jose, CA). Lymphocyte gates were established using CD45brt⁺ fluorescence and low Side Scatter for the Multitest™ tube and Forward Scatter/ Side Scatter with a sequential gate on CD4brt⁺ or CD8brt⁺ cells for markers of lymphocyte activation and maturation. A minimum of 2500 events for each gate was acquired. Activated subsets are defined as CD38⁺HLA-DR⁺, naïve subsets as CD62L⁺CD45RA⁺, and memory subsets as CD62L⁻CD45RA⁺, CD62L⁺CD45RA⁻, and CD62L⁻CD45RA⁻.

Human *TCRD* sjTREC Assay

Quantification of signal joint (sj) T cell receptor delta (*TCRD*) excision circles (TRECs) in isolated CD4⁺ and CD8⁺ T-cells was performed by real-time quantitative-PCR using the 5'-nuclease (TaqMan) assay with an ABI7700 system (Perkin-Elmer, Norwalk, CT) described by Douek et al¹¹. PBMC were separated into CD4⁺ and CD8⁺ populations using MACS magnetic microbeads (Miltenyi-Biotech, Auburn, CA). Cells are then lysed in 100µg/ml proteinase K (Boehringer, Indianapolis, IN) for 1 hour at 56°C, then 10 minutes at 95°C, at 10⁷ cells/ml. Real-time quantitative-PCR was performed on 5µl of cell lysate (equivalent to 50,000 cells) with the primers: cacatcccttcaacctgct and gccagctgcagggttagg, and probe FAM-acacctctggttttgtaaagggtgccact-TAMRA (MegaBases, Chicago, IL). PCR reactions contained 0.5U Platinum taq polymerase (Gibco, Grand Island, NY), 3.5 mM MgCl₂, 0.2 mM dNTPs, 500 nM each primer, 150 nM probe and Blue Dye-636 reference dye (MegaBases). Amplification conditions are 95° for 5 minutes then 40 cycles of 95° for 30 seconds and 60° for 1 minute. A standard curve of *TCRD* sjTREC plasmid DNA (1x10⁷ - 100 molecules) was plotted and sjTREC values for samples calculated by the ABI7700 software. Samples were analyzed in duplicate. Molecules of TRECs were determined in cell lysates equivalent to 50,000 cells.

ELISA

Antigen-specific end-point antibody titers were determined using ELISA as previously described^{12,13} except that black ELISA plates were used (black microflour 2 ELISA plates, DYNEX, Thermo Labsystems, Franklin, MA) and the fluorescent alkaline phosphatase substrate Attophos (Roche Molecular Biochemicals, Indianapolis, IN) was used. Fluorescent ELISA plates were read on a FluoroCount fluorescent plate reader (Packard Instrument Company, Meriden, CT). Samples were considered positive for antigen-specific antibody when the relative light units (RLU) for the sample dilution was 3 fold higher than the RLU for a naïve sample at the same dilution. HIV antigens were coated to the ELISA plates at 5 ug/ml.

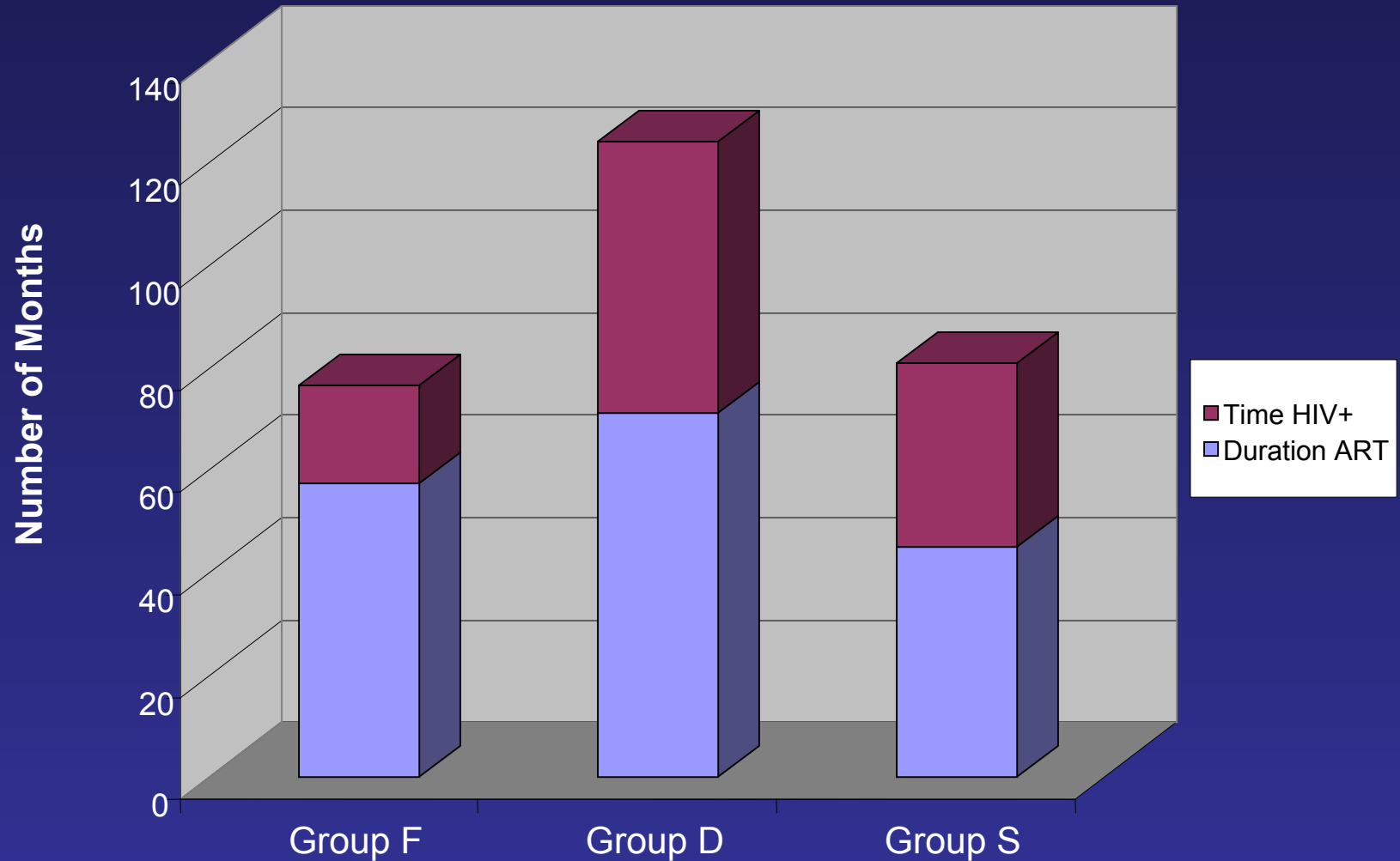
Clinical History

	Group F	Group D	Group S
Age	45 (40-49)	46 (37-51)	39 (38-41)
Gender	8M, 2F	7M, 3F	7M, 3F
Ethnicity*	6AA, 4W	1AA, 9W	6AA, 4W
Baseline/Off Rx VL* (copies/mL)	170203 [n=4] (145K-204K)	63607 [n=6] (31414-144K)	64255 [n=9] (43237-180K)
CD4 ⁺ Nadir (cells/mm ³)*	20 [n=6] (3-34)	160 [n=7] (102-292)	124 [n=9] (19-234)
CD4 ⁺ Nadir (%)*	2 (0-3)	11 (8-15)	14 (2-17)

*Group F vs. D p<0.05

HIV History

(Height of bar = total time HIV+)



CD4⁺/VL Data at Time of Study Visit

[median (IQR)]

	Group F	Group D	Group S
VL (copies/mL)	33855 (20516-74273)	1506 (1134-3400)	50 (50-50)
CD4 ⁺ (cells/mm ³)	133 (49-213)	480 (375-570)	454 (439-788)
CD4 ⁺ (%)	9.3 (5.8-13.3)	21.0 (19.1-24.8)	28.8 (23.8-34.9)

CD4⁺ and CD8⁺ TRECs

[median TRECs/100,000 cells (IQR)]

	Group F	Group D	Group S	F vs. D p-value	S vs. D p-value
CD4 ⁺	685 (43-1700)	256 (0-1852)	1144 (263-3497)	0.9	0.2
CD8 ⁺	594 (0-822)	109 (0-819)	581 (68-2575)	0.7	0.2

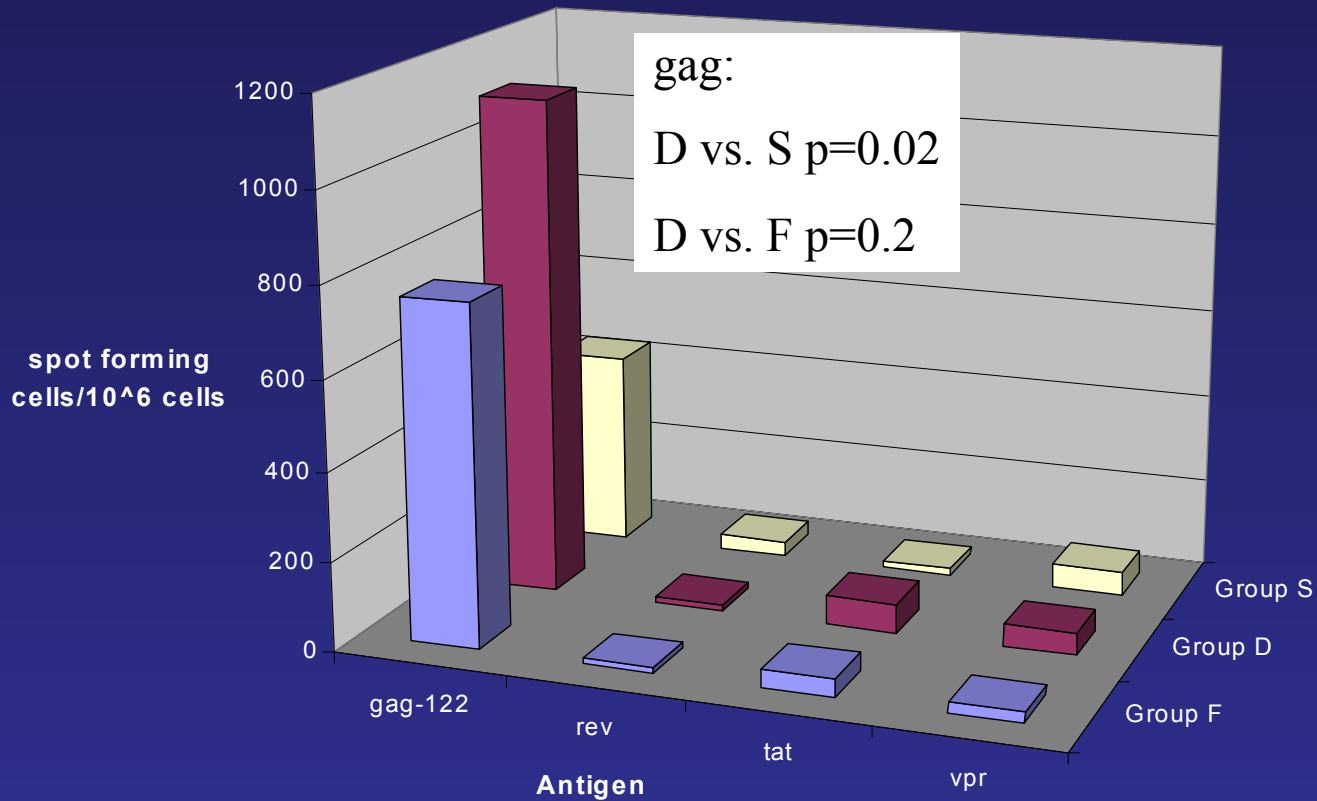
Serum IgG Antibodies

[median (IQR) log₂ endpoint titers]

Antigen	Group F	Group D	Group S
DP31 peptide	15 (13-16)	14 (13-16)	15 (10-16)
MNV3 peptide	9 (7-11)	10 (8-10)	10 (9-12)
gp120	15 (13-15)	15 (14-16)	14 (13-15)

IgM and IgA responses were rare and of low titer.

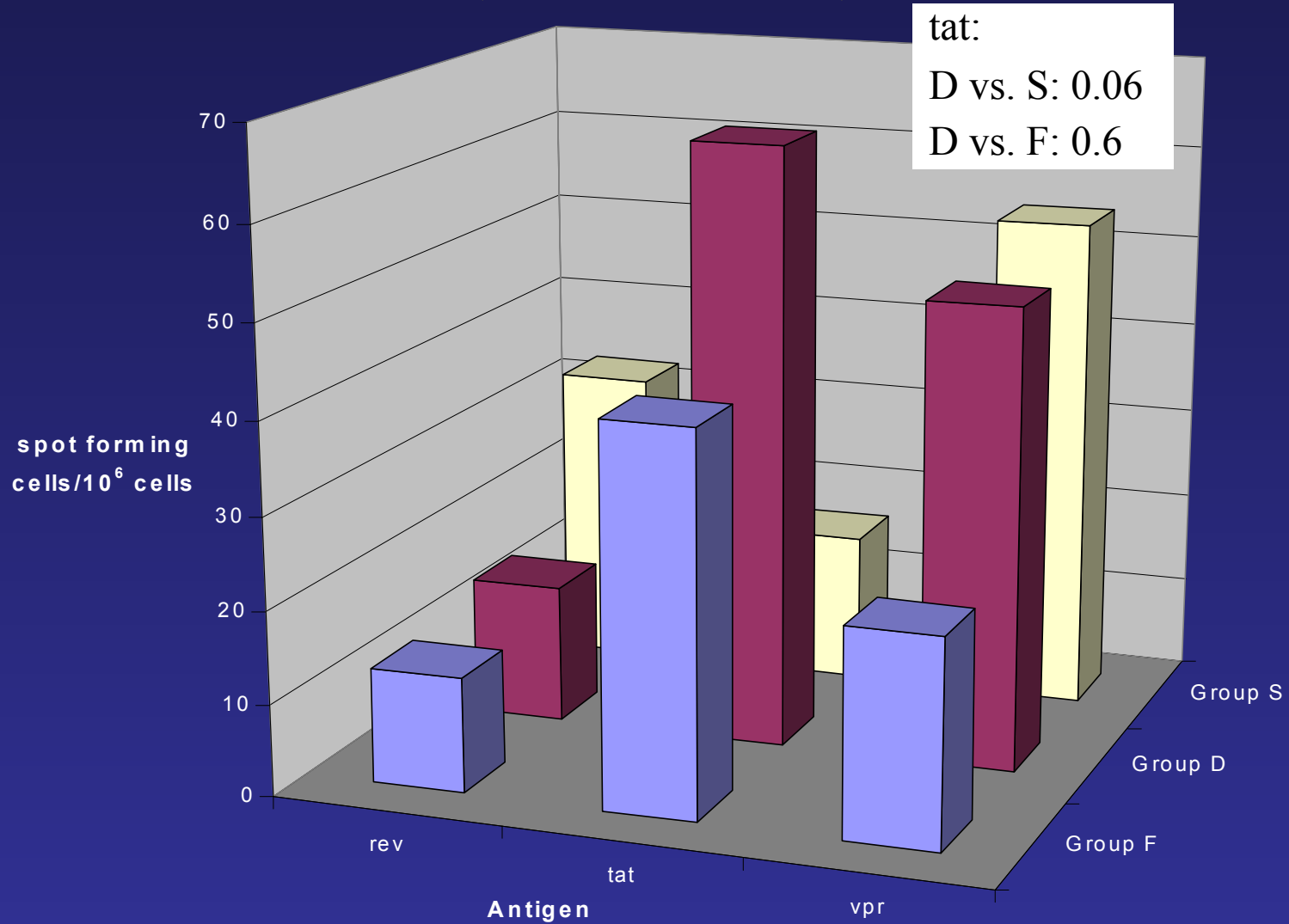
IFN- γ ELISpot (CD4⁺ and CD8⁺)



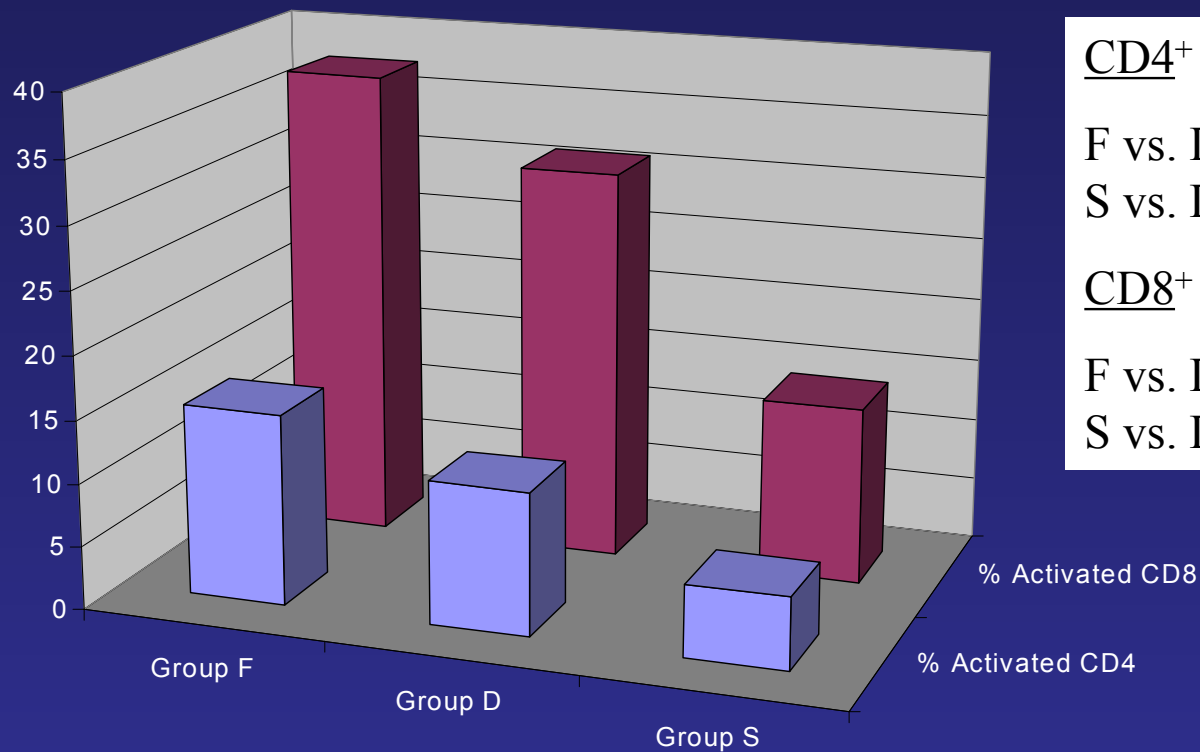
A detectable CD4⁺ response to gag was shown in 5/9 D subjects, 3/8 F, and 0/10 S.

IFN- γ ELISpot (continued)

(CD4⁺ and CD8⁺)



Cellular Activation as Measured by CD38⁺HLA-DR⁺ Expression



CD4⁺ Cells:

F vs. D p=0.06

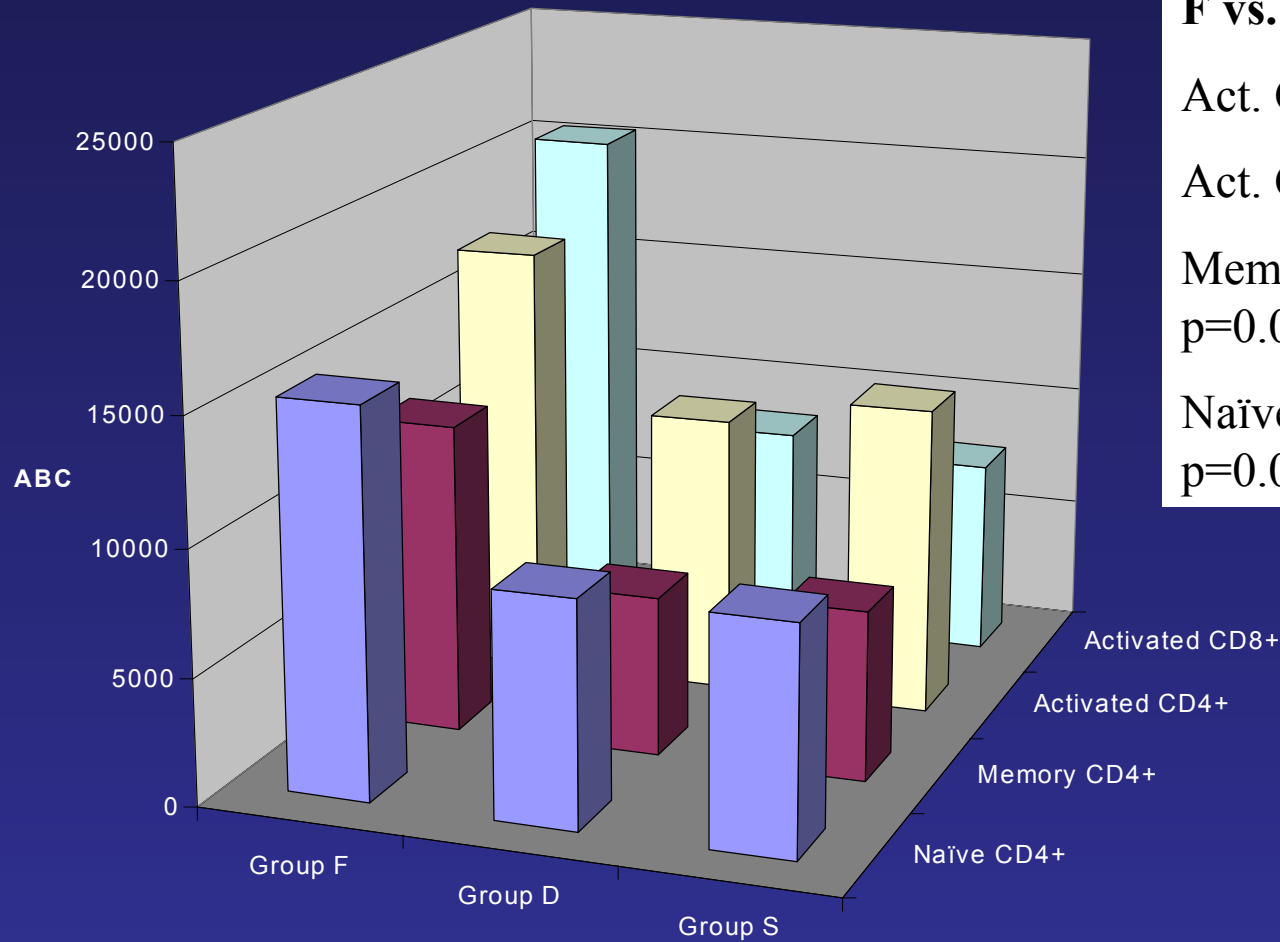
S vs. D p=0.06

CD8⁺ Cells:

F vs. D p=0.08

S vs. D p=0.02

CD38⁺ Antibodies Bound per Cell (ABC)



F vs. D:

Act. CD8⁺ p=0.008

Act. CD4⁺ p=0.04

Memory CD4⁺
p=0.005

Naïve CD4⁺
p=0.01

Conclusions

- Compared to subjects failing HAART, subjects with a discordant response have:
 - lower baseline VL
 - higher nadir CD4⁺
 - decreased immune activation
- Compared to subjects with full viral suppression, subjects with a discordant response have:
 - greater levels of immune activation
 - enhanced HIV-directed CD4⁺ T-helper and CD8⁺ responses to gag and tat
- Study Limitations:
 - small sample size
 - possible selection bias
 - subjects not all on same regimen
 - multiple comparisons

Potential Mechanism of Discordant CD4⁺/VL Responses

- Incompletely suppressive HAART regimens select for resistance mutations that decrease viral replicative capacity, leading to persistent low-level viremia (see Poster 483M)
- Diminished viral replication decreases cellular activation, reducing pace of CD4⁺ destruction
- At least partial immunologic recovery occurs despite low level viral replication
- Immunologic recognition and generation of HIV-specific immune responses helps maintain control of HIV replication

Implications of Findings

- Fundamental goals of HIV treatment are clinical and immunologic well-being
- Triggers for changing antiretroviral therapy need to focus on more than just VL
- Long term studies assessing treatment strategies required (ACTG 5115)
- Appropriate interpretation of viral resistance and replicative capacity testing require clinical context

Future Directions

- One year follow-up study of this cohort to assess CD4⁺/VL changes, genotype/phenotype evolution, replicative capacity, and immune function
- Assessment of these factors in patients on an NNRTI-based regimen
- Prospective studies needed to determine predictive factors and proper clinical management of patients with a discordant response to their treatment regimen

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References

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