Beneficial Effects of a Switch to a Lopinavir/ritonavir-Containing Regimen for Patients with Partial or No Immune Reconstitution with Highly Active Antiretroviral Therapy Despite Complete Viral Suppression

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Abstract

The purpose of this study was to determine if switching to an Lopinavir/ritonavir (LPV/r)-containing regimen resulted in greater immune reconstitution in patients with immunologic failure despite complete viral suppression with highly active antiretroviral therapy (HAART). Twenty patients with partial or no immune response to HAART despite viral suppression were enrolled. Ten were randomized to stay on their current regimen and 10 were randomized to LPV/r plus their current NRTI backbone. T cell subsets, *ex vivo* apoptosis, and the percent of circulating cells with detectable intracellular HIV-1 RNA were measured. The mean increase in $CD4^+$ count at 6 months was $116/mm^3$ (172–288) for the LPV/r-containing arm versus $32/mm^3$ (264–296) for continuation regimens ($p = 0.03$). The number of patients with an increase $\geq 50\,cells/mm^3$ was also greater in the LPV/r arm (7/9 versus 2/10, $p = 0.01$). This paralleled a decrease in *ex vivo* apoptosis of naive $CD4^+$ T cells at 6 months (21.7–11.0% for the LPV/r arm versus 17.3–18.9% for the continuation arm, $p = 0.04$) and memory cells (21.1–14.1% for LPV/r versus 20.2–17.9% for continuation arm, NSS). Switching patients to an LPV/r-containing regimen improved $CD4^+$ counts in patients with prior immunologic failure, and this may be due to an effect of LPV/r on apoptosis.

Introduction

HIGHLY ACTIVE ANTIRETROVIRAL therapy (HAART) results in both a reduction in viral load and an increase in the absolute number of $CD4^+$ T cells, even in patients with advanced disease.[1] Discordant immune responses, however, occur, including poor immune responses despite adequate viral suppression, and this is associated with excess morbidity and mortality.[2–4] There is some evidence that protease inhibitors (PIs) may have beneficial effects on immune reconstitution that are independent of their antiretroviral effects. This may in part be due to inhibition of T cell apoptosis.[5–7] Although there are treatment guidelines for patients with virologic failure to HAART, there are currently no guidelines that recommend changes in therapy based on immunologic failure. The Department of Health and Human Services (DHHS) treatment guidelines address immunologic failure, and recommend evaluation for other causes of immune deficiency, including coinfection with other immunomodulating viruses.[8] No changes in antiretroviral therapy are currently recommended except to consider changing one of the agents in patients receiving both didanosine (ddI) and tenofovir, a combination that has been associated with poor $CD4^+$ recovery. Intensification and/or immune-based therapy with interleukin (IL)-2 are not recommended. It is important to develop new strategies to manage patients with immunologic failure or only partial immune recovery.

PIs have been associated with immunologic benefits independent of viral suppression. A number of studies suggest that PI-containing regimens are associated with greater increases in $CD4^+$ counts than nonnucleoside reverse transcriptase inhibitor (NNRTI)-based regimens, despite comparable viral suppression.[9–11] Naive patients treated with Lopinavir/ritonavir (LPV/r) have seen normalization of $CD4^+$ counts, including those patients who initiate therapy with a $CD4^+$ count $<50/mm^3$.[12] This is a group that normally does not attain a normalization of $CD4^+$ count, even with complete viral suppression.[13,14]

Few data are available, however, on the benefits of PIs for treatment-experienced patients with suboptimal immunologic

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responses or immunologic failure. The purpose of this study was to determine if a switch to an LPV/r-containing regimen while continuing the current two-drug NRTI backbone would result in improved immune response to therapy compared to continuation of the current regimen. Our data suggest this may be an effective strategy for improving immune recovery in treatment-experienced patients with partial or no immune response to HAART despite complete viral suppression.

Materials and Methods

Study design

This was a prospective, open-label, randomized controlled trial. Patients who had been receiving a stable triple-drug HAART regimen with complete viral suppression for >6 months were eligible for the study. Patients were enrolled from the outpatient clinics at the University of Chicago and the University of Illinois, with approval from the Institutional Review Board at each institution. We grouped patients according to their baseline absolute $CD4^+$ lymphocyte counts before the initiation of HAART as follows: group 1, $CD4^+ < 100/mm^3$; group 2, $100–199/mm^3$; group 3, $200–399/mm^3$, group 4, $400–699/mm^3$; and group 5, $\geq 700/mm^3$. We defined immune responses as follows: complete immune responders have $CD4^+$ counts increase to $>700/mm^3$ after initiation of HAART; partial immune responders have an increase in $CD4^+$ count of >50% and improvement by at least one immunological grouping; and all other patients are considered nonresponders. The initial goal was to enroll 40 patients with viral load $<50/mm^{33}$ for >6 months on a stable HAART regimen without a complete $CD4^+$ immune response, with 20 immune nonresponders and 20 partial responders being offered enrollment into the study. In the end, the decision was made to close enrollment due to slow accrual, with 20 patients meeting the study criteria consenting to participate in the study.

Patients with partial immune or no immune response were randomized to continue their present regimen or switch to an LPV/r-containing regimen by replacing their current PI or NNRTI while continuing the two NRTI backbone of the current regimen. If patients were on a triple nucleoside regimen (AZT/3TC/ABC, Trizivir in all cases), the ABC was discontinued so they remained on a three-drug regimen.

Endpoints

Primary endpoint. Immune reconstitution was measured as an increase in absolute $CD4^+$ T lymphocyte count after 1, 3, and 6 months of therapy.

Secondary endpoints. (1) Rates of *ex vivo* T cell apoptosis, both memory and naive $CD4^+$ T cells, and $CD8^+$ T cells, (2) activation measured as the percent $CD4^+$ cells expressing CD38, (3) low-level viral replication measured as the percent cells with detectable intracellular HIV-1 RNA in different peripheral blood mononuclear cell (PBMC) subsets, (4) clinical HIV-related events, and (5) virologic failure defined as HIV RNA >2000 copies/ml.

In addition to the subjects randomized to a treatment arm, we did studies of *ex vivo* apoptosis in a group of HIV-negative controls and a group of HIV-infected subjects with complete

immune response, $CD4^+ > 700/mm^3$, on HAART. This included five subjects on an LPV/r-containing regimen and five subjects on another standard three-drug HAART regimen. Expression of CD38 and intracellular viral load were also examined in the complete immune responder group.

The interventional portion of the study was 6 months in duration, but we continued to collect data on increases in $CD4^+$ counts every 3 months as long as the patient remained on the same HAART regimen.

Apoptosis assays

T cell subsets (naive $CD4^+$ T cells, $CD4^+CD45RA^+$; memory $CD4^+$ T cells, $CD4^+CD45RO^+$; and CD8 T cells) were isolated by sorting with magnetic beads. Cells were kept in culture in RPMI + L-glutamine at 37°C in 5% CO_2 for 3 days. At that time cells were stained with propidium iodide and the percent apoptotic cells was determined by flow cytometry.[15]

Intracellular viral load

The percent of cells containing HIV-1 RNA was determined by flow cytometry for the various T cells subsets using the ViroTech HIV-1 Flow Cytometry Assay.[16] Specimens of EDTA-anticoagulated whole blood were shipped overnight to Esoterix, Inc. for analysis. Briefly, isolated PBMCs were stained with cell surface-directed monoclonal antibodies for identification of various PBMC subtype populations for 20 min at room temperature (RT), washed, and then resuspended. Cells were then incubated with permeabilization reagent for 1 h at RT in the dark. After washing, hybridization probe cocktail was added to detect gag–pol mRNA, and the cells were incubated for 30 min at 43°C. Cells were then washed three times and analyzed by flow cytometry to determine the percent of each cell subtype containing intracellular HIV-1 mRNA.

Statistical analysis

The differences in mean $CD4^+$ count, mean percent increase in $CD4^+$ count, percent $CD4^+CD38$ cells, and percent expressing intracellular HIV-1 RNA were analyzed by the Student's t test. Differences between arms in the number of patients having a >50 cell/mm^3 increase in the absolute $CD4^+$ T cell count cells were analyzed by the Fisher exact test.

Results

Twenty HIV-1-infected patients who met the criteria for suboptimal immune response were enrolled into the study. The baseline characteristics, including the HAART regimens at the time of enrollment, are shown in Table 1. There were no statistically significant differences with respect to age, race, duration of HAART prior to study entry, partial or no immune response between the treatment arms, or baseline absolute $CD4^+$ count at study entry. Although patients were eligible for enrollment if they had viral suppression for 6 months on a stable HAART regimen, most had been on HAART for 2 or more years. The mean duration of HAART prior to study entry was higher for the continuation group, but this difference was not significant and one patient in the continuation arm who had been on HAART for 108 months was entirely responsible for this difference. Nineteen patients completed the study and were included in the analysis. One

TABLE 1. BASELINE DEMOGRAPHIC CHARACTERISTICS OF THE TWO TREATMENT GROUPS

	Switch to LPV/r-containing regimen (N = 10)	Continued current HAART regimen (N = 10)
Mean age (years)	45.5 ± 14.4	40.5 ± 9.6
Gender	8 males	9 males
	2 females	1 female
Mean CD4 T cell count/mm^3	172 ± 89 (range 90–249)	264 ± 106 (range 82–451)
Immune response at enrollment	5 nonresponders	5 nonresponders
	5 partial responders	5 partial responders
Duration of HAART prior to study entry (months)	28.8 ± 23.4 (range 7–65)	38.3 ± 28.8 (range 12–108)
HAART regimen at enrollment		
Two NRTIs + NNRTI	7	6
Two NRTIs + PI	2	1
Two NRTIs + boosted PI	0	1
Three NRTIs	1	2

There were no statistically significant differences between the treatment groups by Student's t test.
HAART, highly active antiretroviral therapy.

patient in the LPV/r arm discontinued early because of grade II gastrointestinal symptoms. Of note, none of the patients had viral failure, viral rebound, or a "blip" in viral load at any point in the study.

Patients who were switched to an LPV/r-containing regimen had a significantly greater increase in their absolute CD4$^+$ count at 6 months than the continuation group, 116 ± 108 cells/mm^3 versus 32 ± 49 cells/mm^3, $p = 0.03$ (see Fig. 1). There were no significant differences at earlier time points. Seven of nine patients in the LPV/r arm versus two of 10 patients in the continuation arm had a ≥50 cell/mm^3 in-

crease in absolute CD4$^+$ count at 6 months ($p = 0.02$, Fisher exact test). Even if the single patient in the LPV/r arm who discontinued therapy due to GI side effects is counted as an immune failure, the immune response for 7 of 10 subjects is still significantly greater ($p = 0.035$, Fisher exact test). There were also trends toward a greater increase in the percent CD4$^+$ cells, percent naive (CD4$^+$45RA$^+$) cells, and absolute naive cells, but none of these measures reached statistical significance.

The increase in absolute CD4$^+$ in the LPV/r arm was associated with a decrease in ex vivo T cell apoptosis. Similar to
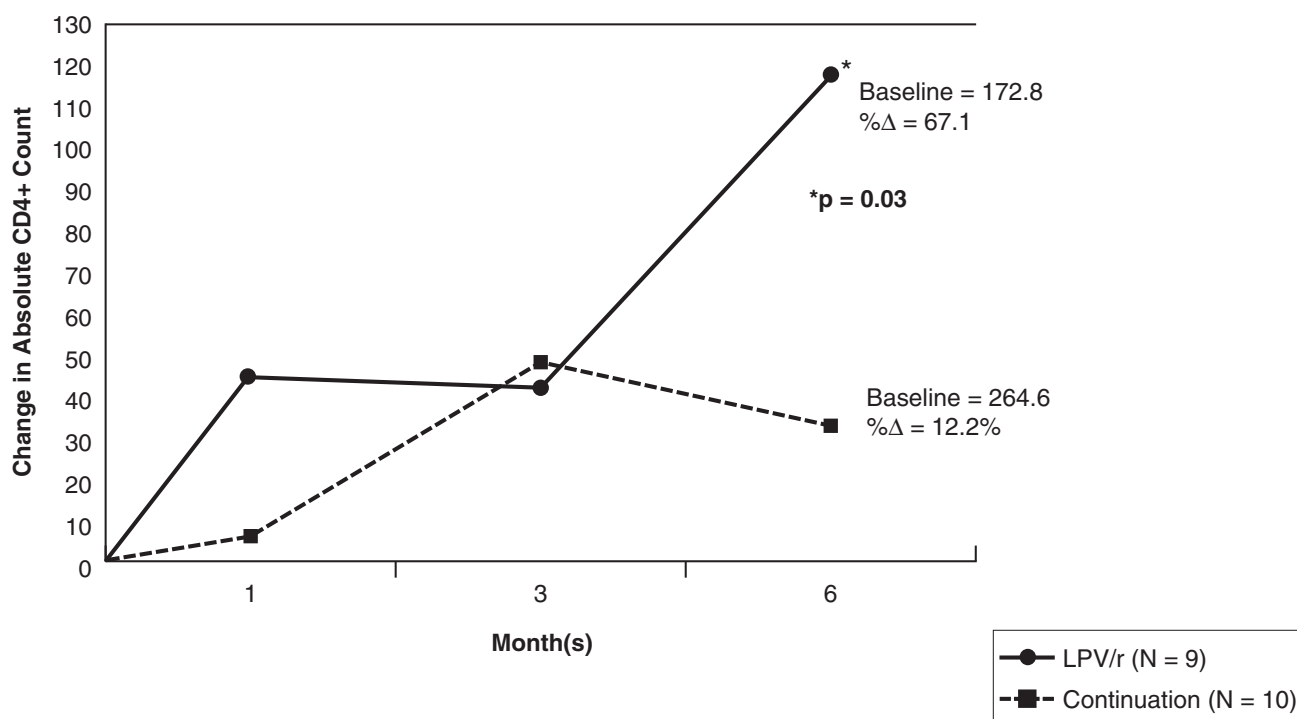

FIG. 1. Change in absolute CD4$^+$ T cell counts for the two treatment groups. The difference in the mean change in absolute CD4$^+$ count at each time point was analyzed by the Student's t test, but reached significance only at 6 months. At 6 months the patients in the LPV/r-containing arm had a mean increase in CD4$^+$ T cell count of 116/mm^3 versus 32/mm^3 in the continuation of current HAART regimen arm. *p = 0.03.

previous studies, the rate of *ex vivo* T cell apoptosis was significantly higher in patients with partial immune recovery or virologic failure compared to HIV-negative controls or the HIV-infected complete immune responder control group (see Fig. 2). Patients in the LPV/r arm had a significant decrease in the proportion of apoptotic naive cells at 72 h *ex vivo* compared to the continuation arm (see Fig. 2a). The change in apoptosis at 6 months was -11.7% for the LPV/r arm versus $+$
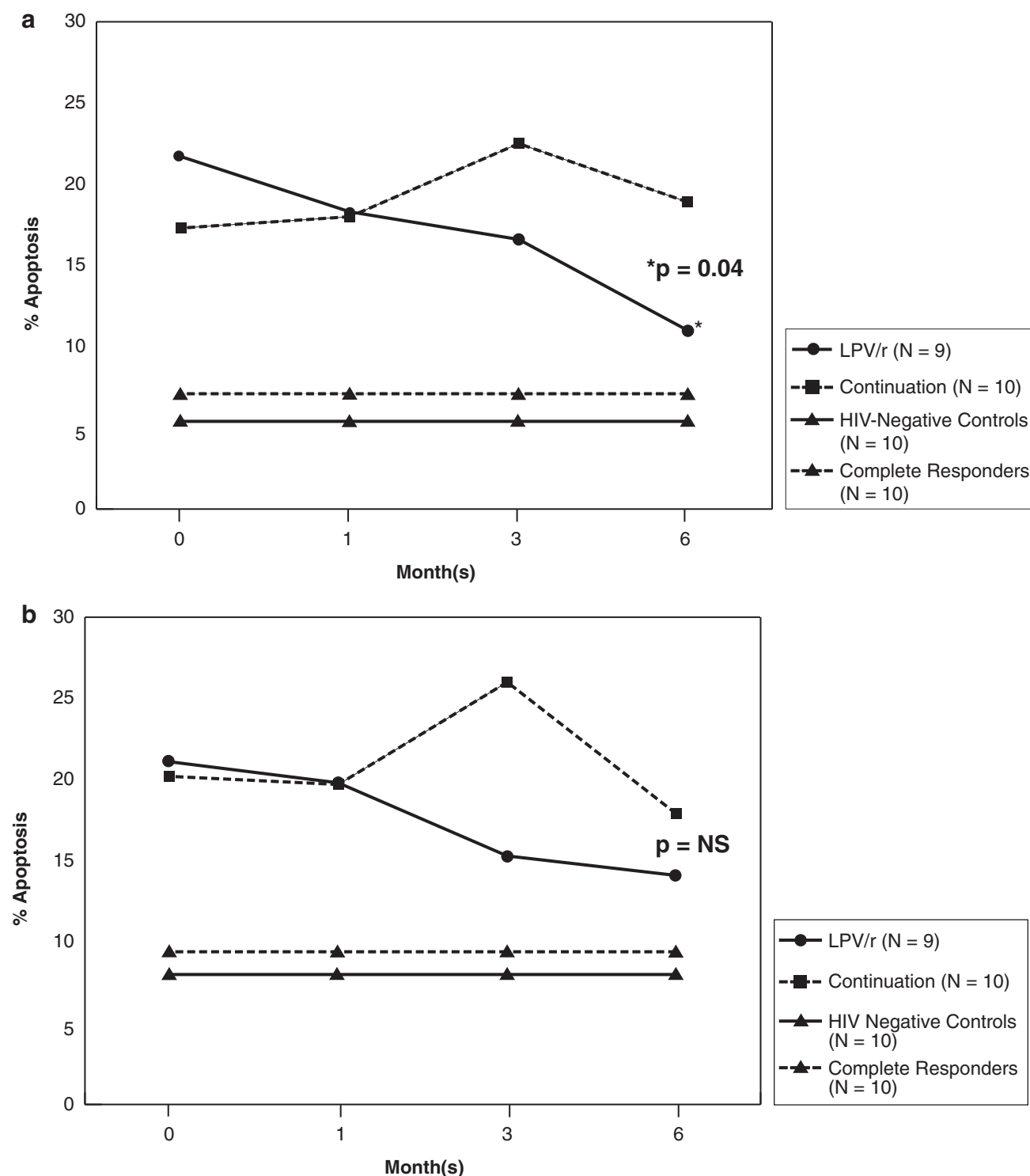

FIG. 2. *Ex vivo* apoptosis of CD4$^+$ T cells, both naive (CD4$^+$CD45RA$^+$) **(a)** and memory (CD4$^+$CD45RO$^+$) **(b)** phenotypes. The *ex vivo* apoptosis for patients in both treatment arms is shown in relation to the mean rate of apoptosis of the HIV-negative controls and a group of HIV-infected complete responders. At 6 months the rate of apoptosis for naive cells in the LPV/r group was significantly decreased compared to the continuation arm and approached the level seen in the control groups (Student's *t* test). There was a trend toward being decreased in the memory cells as well, but this did not reach significance. *p $= 0.04$.

1.6% for the continuation arm ($p = 0.04$, t test). The percent naive CD4$^+$ T cell apoptosis in the LPV/r-containing regimen arm at 6 months (11.0%) was similar to that of the HIV-negative and HIV-infected complete responder control groups, 5.4% and 7.1%, respectively. There also was a trend toward reduction of apoptosis of memory CD4$^+$ T cells in the LPV/r arm, but this did not reach statistical significance (Fig. 2b). There were no significant differences in the change in apoptosis of CD8 T cells between arms or over time (-3.6 ± 8.8 for LPV/r arm versus -2.6 ± 16.0 for continuation arm at 6 months).

We did not note any changes in the expression in CD38 on CD4$^+$ T cells between the treatment groups, either at baseline or over the 6-month study period (see Table 2). Interestingly, baseline CD38 expression on CD4$^+$ T cells was no higher for the partial or nonimmune responders in either arm compared to the HIV-infected complete immune responders. All patients had HIV-1 RNA detected in a portion of their circulating T cells and monocytes, but we did not observe any significant changes in the proportion of T cells or monocytes with detectable HIV-1 RNA over time in either arm. There were also no differences in baseline intracellular HIV-1 RNA between the treatment groups and the complete immune responder control group.

We continued to follow absolute CD4$^+$ counts and viral loads for the subjects after the 6-month study period as long as they remained on the same regimen. In addition, there were nine patients who met inclusion criteria and were offered enrollment, but elected not to participate because they did not want to change regimens or did not want any change to be determined randomly. Although these patients were not enrolled into the interventional study, they did consent to have us follow their CD4$^+$ counts over time as long as their viral load remained <50 copies/ml and they remained on a stable HAART regimen. This group served as a second observational control group to assess immune recovery beyond 6 months. The patients switched to an LPV/r-containing regimen had a continuous rise in their absolute CD4$^+$ counts over time and did not show any plateau out to 3 years (see Fig. 3). In contrast, patients who continued their initial HAART regimens, either as part of the continuation arm of the study or as part of the observational control group, had little improvement in their absolute CD4$^+$ counts over time.

Discussion

Our pilot study demonstrates that a change to an LPV/r-containing regimen can improve immune recovery in patients who have had a suboptimal immune response to HAART despite viral suppression. The goal of HAART is both complete viral suppression and reconstitution of the immune system. Although viral suppression usually leads to improved immune status, there are patients with discordant responses who demonstrate complete viral suppression as measured by plasma viral load but do not have an adequate increase in the CD4$^+$ count.[2–4] There is excess morbidity and mortality for patients with immunologic failure, particularly for those patients whose CD4$^+$ counts do not increase above 200/mm^3. The long-term immune responses to HAART are usually predicted by the viral and immune response at 3–6 months, the rationale for us to enroll and evaluate patients who had inadequate CD4$^+$ T cell responses at a 6-month time point.[17–19] The definition of an inadequate CD4$^+$ T cell increase or immune nonresponse varies. Some investigators have defined this as an increase in CD4$^+$ count <50–100/mm^3 in the first 6–12 months after the initiation of HAART. There are data that suggest that a patient who does not have an increase at this point is unlikely to have adequate CD4$^+$ recovery over time. Across a number of studies, the proportion of patients who have immunologic failure on a suppressive regimen ranges between 10% and 25%.[2–4,20–22] Additionally, a large proportion of patients do not meet the definition of immunologic failure, yet have only partial immune recovery and never achieve a normal absolute CD4$^+$ T cell count. Return to a normal CD4 T cell count may also be clinically relevant and the view of what constitutes an adequate immune response is evolving. In the past, recovery of CD4$^+$ counts to >200/mm^3 was believed to be adequate. However, new data from a number of cohorts suggest that any incremental increase in the CD4$^+$ count up to 500/mm^3 is associated with a reduction in mortality, not only from HIV-defining complications,

TABLE 2. CD4$^+$ CELL ACTIVATION AS MEASURED BY CD38 EXPRESSION AND LOW-LEVEL VIRAL REPLICATION AS MEASURED BY PERCENT CD4$^+$ PERIPHERAL BLOOD MONONUCLEAR CELL SUBSETS POSITIVE FOR INTRACELLULAR HIV-1 RNA

	Switch to LPV/r-containing regimen (N = 9)	Continued current HAART regimen (N = 10)	Complete responder controls (N = 10)
% CD4$^+$CD38$^+$			
Baseline	5.1 ± 1.8	5.4 ± 2.3	8.8 ± 4.5
6 months	6.0 ± 2.8	5.7 ± 2.6	NA
Intracellular HIV RNA			
CD4$^+$CD45RA$^+$			
Baseline	5.9 ± 9.7	3.3 ± 5.7	3.3 ± 3.3
6 months	5.9 ± 6.9	4.9 ± 3.7	NA
CD4$^+$CD45RO$^+$			
Baseline	11.0 ± 19.1	8.9 ± 17.2	11.4 ± 20.2
6 months	10.6 ± 13.7	6.0 ± 5.1	NA
Monocytes			
Baseline	11.2 ± 9.0	20.8 ± 22.8	24.2 ± 8.1
6 months	18.5 ± 17.8	10.5 ± 9.0	NA

There were no statistically significant differences between treatment groups or the complete responder control group by Student's t test.
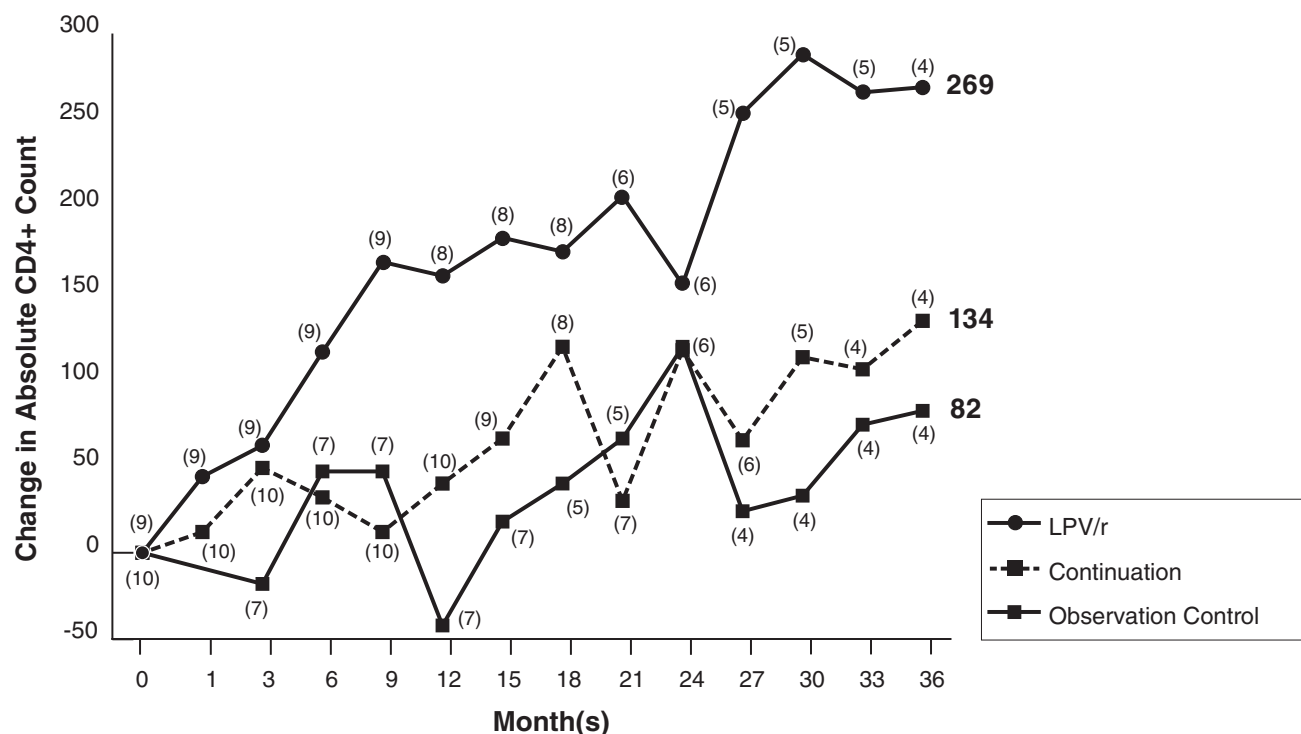
FIG. 3. Increase in absolute CD4$^+$ T cell counts after the 6-month study period. We continued to follow CD4$^+$ T cell counts in the LPV/r and continuation study arms after the 6-month study period ended, as well as the counts for an observational group that met study entry criteria but chose to remain on their same regimen instead of enrolling in the trial. If patients still had an undetectable viral load and remained on the same HAART regimen, they were included in this analysis. The patients remaining on a continuation HAART regimen had little increase in CD4$^+$ T cell counts over time, while the patients in the LPV/r arm continued to increase over time.

but from other conditions including hepatitis, cancer, and cardiovascular disease.[23–25]

The underlying mechanisms of immunologic failure and discordant immune responses are not completely understood. Immune activation is an important part of the pathogenesis of HIV infection, and ongoing immune activation may be one mechanism that potentially contributes to immunologic failure.[26–29] There is the potential that other coinfections blunt the immune recovery despite effective HAART. However, this does not explain most cases of immunologic failure, and in our study there was no evidence of increased immune activation in immunologic failures compared to the complete immune responder control group, at least as measured by T cell expression of the T cell activation marker CD38. There is also a search underway for the genetic basis of immunologic success or failure. A substudy of ACTG 5001 looked at single nucleotide polymorphisms (SNPs) in a number of immune response genes in patients with undetectable plasma viral load at 48 weeks in ACTG studies of various HAART regimens. So far there have been some associations between certain SNPs and immune response, but the data are very preliminary.[30]

There is also evidence, that despite complete viral suppression, immunologic failure is associated with persistently accelerated T cell apoptosis.[31,32] Our group and others have shown that patients with complete immune recovery have levels of apoptosis similar to HIV-negative controls, while immunologic failures have very high rates of *ex vivo* T cell

death, and patients with only partial CD4$^+$ recovery have intermediate rates of *ex vivo* apoptosis.[31] There is also a significant body of literature that suggests that inadequate thymic function or inability to recover thymic function may be the major barrier to immune reconstitution.[33–35]

Although there are numerous recommendations on the management of patients with virologic failure on HAART, there is little guidance for clinicians who have patients with immunologic failure despite complete viral suppression.[7] Immune-based therapy is one approach, and there are a number of studies of immune-based therapy with IL-2 that have shown sustained CD4$^+$ T cell increases, including increases in patients with prior inadequate immune responses.[36,37] Recent data from the ESPRIT (Evaluation of Subcutaneous Proleukin in a Randomized International Trial) and SILCAAT (Subcutaneous IL-2 in patients with Low CD4 Counts under Active Antiretroviral Therapy) trials, however, do not show that immune function or clinical outcomes are actually improved despite increases in the CD4$^+$ count with IL-2.[38] The lack of clinical benefit along with the side effect profile and the need for injections make IL-2 treatment difficult and currently not recommended. The same is true for other immune-based therapies, such as GM-CSF, IL-7, and human growth hormone.[39]

Although a change in antiretroviral regimen seems reasonable and was recommended in earlier panel guidelines, the current DHHS guidelines do not recommend any change in antiretroviral therapy in patients with immunologic failure

despite viral suppression, even in patients with $CD4^+$ counts $<200/mm^3$. This is mainly due to the lack of studies that show the benefit of any particular treatment strategy for immunologic failure. The current and previous DHHS guidelines warn that zidovudine or tenofovir in combination with didanosine can suppress bone marrow and leukocyte counts, including $CD4^+$ counts. There are no prospective studies demonstrating that switching out these agents is associated with improved immune recovery, and if didanosine is given at a reduced dose when combined with tenofovir, there appears to be no detrimental effect on $CD4^+$ counts.

Attempts at improving immune reconstitution by intensification, theoretically reducing ongoing low-level viral replication, have been reported, but the results have been conflicting.[40] Currently there are no convincing data that intensification in patients with an undetectable plasma viral load is beneficial. One variable clearly associated with immune recovery is the baseline $CD4^+$ count. On average, patients who start HAART at a $CD4^+$ count $<200/mm^3$ are less likely to ever reach a normal $CD4^+$ count, even after years of treatment.[13,14] Earlier initiation of therapy could potentially improve immune outcomes, but this is a moot point for the majority of patients presenting with advanced disease.

The rationale for studying whether a switch to a boosted PI could benefit patients with suboptimal $CD4^+$ recovery was based on several previous studies. Other studies have suggested that PIs have beneficial immune effects that are independent of viral suppression. Deeks and co-workers observed long ago that patients receiving a PI had stable or improved $CD4^+$ counts despite persistent viremia.[10] The comprehensive meta-analysis by Bartlett and co-workers has shown that boosted PI regimens have superior immunologic outcomes compared to NNRTI-based regimens, unboosted PI-based regimens, or triple nucleoside regimens.[11] This was despite an identical proportion of patients achieving complete viral suppression in the boosted PI-based and NNRTI-based regimens. Studies of LPV/r specifically have suggested a beneficial immune effect independent of viral suppression and superior $CD4^+$ T cell increases compared to other HAART regimens.[41] In ACTG 5142, a head-to head study between a boosted PI-based regimen (LPV/r) and an NNRTI-based regimen (EFV) in naive patients, LPV/r was associated with a significantly greater $CD4^+$ increase versus EFV, even though the proportion of patients with complete viral suppression was greater in the EFV arm.[42] In the 720 study of LPV/r in combination with two NRTIs in naive patients, the average increase in $CD4^+$ count after 6 years of therapy was greater than $500\,cells/mm^3$, even in patients who started therapy with a $CD4^+$ count $<50/mm^3$, a level usually associated with suboptimal immune recovery.[12]

The mechanism of improved immune outcomes with boosted PIs may relate to their ability to decrease T cell death independent of their antiviral effects.[7,43–46] A number of studies have shown that PIs can improve the survival of T cells *in vitro*, both T cells isolated from HIV-infected patients and HIV-negative controls.[7] Badley and co-workers have shown that PIs bind to adenine nucleotide translocator (ANT), a pore protein in the mitochondrial membrane.[45] This binding stabilizes the mitochondrial membrane and reduces both spontaneous and induced T cell apoptosis. Of note, this is a class effect seen with most PIs with the exception of atazanavir, which does not bind to ANT and does not inhibit T cell

apoptosis *in vitro*.[46] The effect is also seen with other cell types and this has generated interest in the use of PIs for inhibition of apoptosis in other conditions, including sepsis and hepatitis.[47]

Our pilot study demonstrates that a change to an LPV/r-containing regimen can improve immune reconstitution measured by increases in the absolute $CD4^+$ T cell count in treatment-experienced patients with only partial immune recovery or immunologic failure despite an effective, suppressive regimen. It is significant that the change to an LPV/r-containing regimen increased the mean $CD4^+$ count from $172/mm^3$ to $288/mm^3$ in 6 months. The mean baseline $CD4^+$ count was lower for the LPV/r arm, but the difference was not statistically significant. Although the absolute $CD4^+$ counts at 6 months were similar for both arms, $CD4^+$ counts continued to steadily increase after 6 months for patients on LPV/r, indicating the increase in the first 6 months was not just a regression to the mean. An increase in $CD4^+$ count $>200/mm^3$ in patients with immunologic failure also has clinical relevance, as shown in studies of discontinuation of prophylaxis for *Pneumocystis jiroveci* pneumonia. The exact mechanism of this effect is unknown, but the increase in $CD4^+$ T cell count was associated with a decrease in apoptosis of naive $CD4^+$ T cells. The effect of a boosted PI such as LPV/r may reduce peripheral T cell death, enhance positive selection in the thymus by reducing thymocyte apoptosis, or both. We may speculate that positive selection in the thymus may not be an all-or-none phenomenon. If cells do emigrate from the thymus, but have not been adequately "positively-selected," they may undergo accelerated apoptosis. Increased survival of naive cells may lead to improved $CD4^+$ T cell recovery. A surprising observation is that both the decline in naive T cell death and increases in absolute $CD4^+$ counts did not become significant until after 6 months, while an antiapoptotic effect would be expected to occur immediately. This delay in immune recovery was seen in the ACTG 5142 study as well, in fact, the significantly greater increase in the LPV/r arm became statistically significant only at 96 weeks.[43] Another important observation from our study is that not every patient had an immunologic response after the switch to the LPV/r-containing arm, with two patients having a $<50\,cell/mm^3$ increase in the absolute CD4 count. Some patients have a barrier to immune reconstitution that cannot be overcome by a switch to LPV/r or another potent boosted PI-based regimen. We did not evaluate thymic function or $CD4^+$ T cell function in this study, but future studies should evaluate the effects of boosted PIs on thymic function as well as the effect of PIs on the function of T cells rather than absolute numbers of cells alone.

It is noteworthy that we did not find any increase in CD38 expression at baseline compared to complete immune responders and HIV-negative controls, suggesting that not all immunologic failure is due to ongoing immune activation. We also did not see any differences in intracellular viral loads at baseline or changes over the course of the study in either arm. This suggests that differences in immune recovery are not due to low-level viral replication and an intensification effect of LPV/r. Admittedly, however, measuring intracellular viral load has not been validated as an accurate measure of low-level viral replication in patients with undetectable plasma viral loads. Unfortunately we did not study low level replication with an ultrasensitive viral load assay. It is still possible that the improved immune recovery in the LPV/r arm of our

study was due to enhanced antiviral activity or a higher barrier to resistance.

In summary, patients with only partial or no immune response after 6 months or more of effective HAART therapy may benefit from a switch to an LPV/r-containing regimen. The greater increase in CD4$^+$ count is associated with a decrease in the rate of *ex vivo* CD4$^+$-naive T cell apoptosis. Patients switched to an LPV/r-containing regimen increased their CD4$^+$ count from 172 to 288/mm^3, a clinically relevant change. Larger prospective studies are needed to confirm these results and further determine the mechanisms that result in improved immune recovery with LPV/r.

Acknowledgments

Author Disclosure Statement

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