Introduction

The 16th Conference on Retroviruses and Opportunistic Infections (16th CROI) was held in Montreal from February 8-11, 2009. This conference provided some significant new insights into HIV therapeutics, the most important of which are briefly summarized in this newsletter.

When Should Antiretroviral Therapy be Initiated?

Until recently, for asymptomatic patients with established disease, providers generally started antiretroviral therapy (ART) when the CD4+ count fell below 350 cells/mm³. However, the DHHS and IAS-USA treatment guidelines now recommend individualizing the decision to initiate ART for those with higher CD4+ counts, rather than generally deferring therapy as older versions of these guidelines suggested. (DHHS Guidelines, November 3, 2008 http://aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf; Hammer S, et al. JAMA 2008;300(5):555-570)

These changes result, in part, from observational cohort data and some clinical trial data indicating lower rates of mortality, opportunistic infections (OIs), and events not traditionally associated with HIV infection – such as cardiac, liver, and renal disease – when ART is started at higher CD4+ counts. At CROI, new data from cohort studies not only confirm the clinical benefit of treating patients with CD4+ counts between 350-500 cells/mm³, but push the envelope further, documenting a survival benefit for patients treated with CD4+ counts >500 cells/mm³.

Increased Risk of AIDS and Death if ART Delayed until CD4+ <350 cells/mm³

The Antiretroviral Therapy Cohort Collaboration (ART-CC) analyzed time to clinical AIDS diagnoses and death in patients participating in 15 cohorts who began ART with CD4+ counts <350 cells/mm³ after 1997. (Sterne J, et al. Abst. 72LB) Because they did not have data on patients who deferred therapy, and because clinical events could have occurred during the period of time from a patient’s first evaluation until therapy was begun, the investigators turned to data on 21,247 patients from 7 cohorts collected prior to 1997 in order to model this lead time bias and unseen clinical events. As shown in Table 1, the effect of immediate versus deferred therapy was compared across ranges of CD4+ counts of 100 cells/mm³, and there was a significant increase in risk of AIDS and death when therapy was delayed until patients CD4+ counts fell below 350 cells/mm³ compared to earlier treatment.

Table 1. Hazard ratios for AIDS or Death comparing deferred therapy to a lower CD4+ range with initial therapy in higher range

<table>
<thead>
<tr>
<th>Starting therapy in higher range (CD4+ count, cells/mm³)</th>
<th>Starting therapy to lower range (CD4+ count, cells/mm³)</th>
<th>Hazard Ratio (95% CI)</th>
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<tr>
<td>451 – 550</td>
<td>351 – 450</td>
<td>0.99 (0.76 – 1.29)</td>
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<td>351 – 450</td>
<td>251 – 350</td>
<td>1.28 (1.04 – 1.57)</td>
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<td>301 – 400</td>
<td>201 – 300</td>
<td>1.34 (1.12 – 1.61)</td>
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Supported by an educational grant from Merck & Co., Inc. This coverage is not sanctioned by the conference organizers and is not an official part of the conference proceedings.
Unlike the previously reported NA-ACCord data (Kitahata M, et al. 48th ICAAC/46th IDSA, Abst. H-896b), however, the investigators did not see differences in rates of death (excluding AIDS-defining conditions) when comparing treatment initiation across 100-cell strata when CD4+ counts were >250 cells/mm³.

**Increased Risk of Death if ART Delayed until CD4+ <500 cells/mm³**

At this conference the NA-ACCord group extended their analysis beyond the earlier report by comparing survival in patients who initiated or deferred ART with CD4+ counts >500 cells/mm³. (Kitahata M, et al. Abst. 71) The investigators used an intent to treat (ITT) approach, comparing outcomes in patients from 22 US and Canadian cohorts who started on therapy within 18 months of presentation with a CD4+ count >500 cells/mm³ and in patients whose treatment was deferred until 18 months beyond their presentation. Patients who started therapy with CD4+ counts <350 cells/mm³ were excluded from the analysis. The analysis was stratified by cohort and calendar year and adjusted for demographic and clinical characteristics that may bias clinicians’ decisions to prescribe medication earlier versus later. The immediate treatment group included 2,616 patients who started ART with a median CD4+ count of 674 cells/mm³ and the deferred therapy group included 945 patients who started ART with a median CD4+ count of 390 cells/mm³. Delaying ART was associated with a 36% increased risk of death (relative hazard 1.4, 95% CI 1.1 – 1.7; p=0.008). Older age, but not baseline viral load was also associated with an increased risk of death.

Both ART-CC and NA-ACCord argue for earlier initiation of ART for asymptomatic patients than what is generally recommended. However, there is still considerable debate as to whether these data fail to adjust for bias related to factors that influence the decision to start or delay a patient’s therapy. In addition, the causes of death in these studies have not been reported.

**Benefits to ART with Acute HIV Infection**

Controversy also surrounds the decision when to initiate ART in patients with acute HIV infection. Data indicates that while treatment within 6 months of acute infection may preserve HIV-specific CD4+ T cell responses, once therapy is interrupted, this immunologic benefit is lost. (Reviewed in: Hicks C, et al. Clinical and Experimental Immunology 2007;149:211-6) At this conference, data from the Dutch Primo-SHM cohort suggests that patients with acute HIV infection may benefit from a short course of therapy followed by treatment interruption. (Steingrover R, et al. Abst. 70bLB) Investigators followed 141 patients with acute infection or recent infection within the previous 180 days, 47 of whom were untreated, and 55 of whom received a median of 28 weeks of ART (range 21 -62 weeks) prior to discontinuing therapy. Others remained on therapy or were lost to follow-up. There were no differences in baseline viral load or CD4+ counts between groups of patients who were started on ART or who were untreated. The investigators compared the time to the initiation of ART in the group of untreated patients to the time to re-initiation of ART in the patients who were received the short course of therapy, with the data adjusted for the early treatment period in the group that were initially treated. As shown in Figure 1, the majority of patients who were treated and then interrupted ART were able to remain off therapy after more than 200 weeks of follow-up, while most of the patients who were untreated during primary HIV infection eventually started therapy.

**Figure 1. Kaplan Meier plot of the time to (re)start HAART, corrected for the duration of early HAART**

CD4+ counts were similar comparing patients who started ART and those who resumed therapy. Thus, these data suggest that a short course of therapy in patients with acute HIV infection might delay the need to initiate therapy later. What could be the mechanism for this effect? Last year at the 15th CROI this group presented data showing that short course therapy in patients with acute HIV infection lowers the viral load set point. (Steingrover R, et al. 15th CROI, Abst. 698b) Other ongoing studies will help to define the advantages of treating patients with acute HIV infection.
**Benefits to Concurrent ART and TB Treatment**

Finally, when should ART be initiated in patients with tuberculosis? Some clinicians argue for concurrent ART and TB therapy, while others argue that, to avoid potential drug-drug interactions with rifampin, tuberculosis should be treated first and then ART started. A randomized trial from South Africa, that included 645 patients with newly-diagnosed, smear-positive TB, evaluated these differing approaches to care. (Karim S, et al. Abst. 36a) Subjects in the concurrent therapy and sequential therapy groups were similar with respect to demographic characteristics, CD4+ counts, viral loads, and presence of drug-resistant tuberculosis. Subjects in the concurrent therapy and sequential arms started ART 67 days and 261 days after starting TB treatment, respectively. Mortality was 55% lower (HR 0.451, 95% CI 0.26 to 0.79; p = 0.0049) in the concurrent treatment arm compared to the sequential treatment arm.

The take-home message on ART is increasingly clear – if a patient is willing and able to take ART, the patient is more likely to benefit from starting therapy sooner, rather than later, irrespective of his/her clinical circumstance.

**When Should Detectable Viremia be Called Virologic Failure?**

One of the characteristic features of recent major AIDS Clinical Trials Group (ACTG) studies – 5095, 5142, and 5202 – is the use of a 200 copies/mL threshold for virologic failure. This differs from most current industry-sponsored studies, which use a threshold of 50 copies/mL. In an analysis presented at the 16th CROI, ACTG researchers explore the potential implications of using the 200 versus the 50 copies/mL cutoff. (Ribaudo H, et al. Abst. 580)

For the purposes of the analysis, data from two major clinical trials were used – ACTG 5095 (comparison between zidovudine/lamivudine [ZDV/3TC] + efavirenz [EFV], ZDV/3TC/abacavir [ABC] + EFV, and ZDV/3TC/ABC) and ACTG 5142 (comparison between lopinavir/ritonavir [LPV/r] + 2 NRTIs, EFV + 2 NRTIs, and LPV/r + EFV). Both the composite endpoint of time to loss of virologic response (TLOVR, where virologic failures and tolerability discontinuations are considered failures) and a pure virologic failure endpoint were evaluated. An endpoint was considered desirable if it was clinically relevant, i.e., it appropriately triggered a change in therapy to avoid resistance but did not prematurely switch patients who re-suppressed, and easy to use in practice. A false-positive endpoint was defined as occurring when re-suppression occurred without a change in therapy after an endpoint was reached.

Of the 1,479 subjects included in the analysis, 87% achieved a viral load < 200 copies/mL while on randomized ART, with 97% doing so before week 24. By contrast, while 81% reached HIV RNA < 50 copies/mL, only 80% did by week 24. Use of the <50 copies/mL threshold as the criterion for virologic failure was associated with a high rate of false-positive endpoints – 23-32% of these study subjects subsequently achieved an HIV RNA < 50 copies/mL without a change in therapy. When the TLOVR criterion was applied to these ACTG trials, changes in randomized ART (rather than virologic failure) accounted for approximately 50% of the endpoints.

The authors concluded that the ideal virologic endpoint for treatment-naïve studies is one that uses a confirmed viral load > 200 copies/mL on or after week 24, as this avoids premature switching of regimens and still allows most patients to achieve and maintain an HIV RNA < 50 copies/mL. Additionally, a virologic failure criterion of 1,000 copies/mL between weeks 16-24 avoids missing early virologic failures, but does not “overcall” failure. While the analysis presented here is applied to clinical trials subjects, it importantly provides some reassurance for those patients followed in clinical practice who have isolated low-level viremia. This phenomenon might become increasingly common as newer, more-sensitive HIV RNA assays (such as the Roche Taqman and Abbott RealTime) replace older tests in clinical labs. Additionally, maintaining current ART – with ongoing monitoring – when there is a single value between 50 and 200 copies/mL avoids the difficulty of trying to obtain resistance and tropism testing at this low level of viremia.

**Low Level Viral Replication and ARV Intensification in Patients with an Undetectable HIV RNA**

At this meeting, more data were added to a body of literature that documents that low levels of HIV replication occur in patients with a plasma HIV RNA <50 copies/mL. In one study, the transcription-mediated amplification (TMA) assay, with a sensitivity to an HIV RNA <3 copies/mL, was used to measure low levels of HIV RNA replication in the plasma of 180 patients with an HIV RNA <50 copies/mL for a median of 13 months using conventional assays. (Hatano H, et al. Abst. 425) A total of 1,606 assays were performed on 438 specimens. The proportion of subjects with a quantifiable HIV RNA using the TMA assay was 76% to 87% over 7 years of ART, and the proportion of patients with quantifiable viral loads did not change for subjects with virologic suppression for at least 12 months. In addition, there is evidence for HIV...
replication in other viral reservoirs in patients with a plasma HIV RNA <50 copies/mL using conventional assays. HIV was detected in the semen of 4 of 13 (31%) patients with a median of 126 months of plasma HIV RNA <50 copies/mL and in the gastrointestinal lymph tissue of 12 subjects undergoing colonoscopy with an HIV RNA <50 copies/mL for more than 2 years. (Sheth P, et al. Abst. 50; Degray G, et al. Abst. 402)

What then is the source of virus in patients with quantifiable viral loads that are < 50 copies/mL, and can these low HIV RNA levels be reduced further? A prospective intensification study evaluated a therapeutic strategy of adding raltegravir (RAL) 400 mg twice-daily (BID) for 30 days to the ART of 10 subjects with a plasma HIV RNA <50 copies/mL for at least 12 months. (Jones J, et al. Abst. 423b)

Pre- and post-integration viral loads were measured weekly pre-, during, and post-intensification with an assay that can detect <1 copy/mL of HIV RNA, and the patients enrolled had a quantifiable HIV RNA of >1 copy/mL. Median levels of HIV RNA were 0.04 log10 copies/mL pre-intensification, 0.14 log10 copies/mL during intensification, and 0.04 log10 copies/mL post-intensification. Thus, intensification had no effect on low levels of HIV production. This result suggests that the source of low levels of HIV RNA is virus production from long-lived cells, rather than replication in rapidly cycling cells. In addition, these data indicate that antiretroviral therapy alone will not be able to eliminate HIV, no matter how potent the regimen. This latter point was confirmed by other groups. In a study, the size of the latently infected reservoir was measured on multiple occasions before and during treatment with a combination of tenofovir (TDF),/emtricitabine (FTC), ritonavir (RTV)-boosted saquinavir (SQV) and enfuvirtide (ENF). Among 9 subjects who initiated this combination and continued for a minimum of 48 weeks, the number of memory CD4+ T cells harboring replication competent HIV decayed slightly in four subjects and increased slightly in the other five. Therefore, treatment with an aggressive antiretroviral (ARV) combination including 2 NRTIs, a boosted PI and an entry inhibitor had no effect on the size of the latently infected reservoir. (Gandhi R, et al. Abst. 424). The take home message is that HIV eradication will not be possible with ART alone, no matter how aggressive the combination or the mechanisms of action of the individual components of therapy. This goal will only be accomplished through the testing of agents that have the potential to activate HIV replication from cells in which HIV persists.

IL-2 Does NOT Improve Clinical Outcomes

The combination of ART and IL-2 has been demonstrated to result in improved CD4+ counts, but no clinical improvement as measured by reduction in clinical endpoints. (Emery S, et al. Controlled Clinical Trials 2002;23(2):198-220; Levy Y, et al. 9th EACS, Abst. F 14/3) It was still unclear, however, whether the increased levels of CD4+ cells that resulted from IL-2 would, over long-term follow-up, confer greater immunologic health and reduce the risk of clinical disease. As it turns out, they do not.

ESPRIT randomized more than 4,000 patients whose CD4+ count was ≥ 300 cells/mm³ to receive ART only or ART plus IL-2. (Losso M, et al. Abst. 90aLB) The results of the study demonstrated that IL-2 could significantly increase CD4+ cells over time and maintain those increases over a 7 year period; however, that CD4+ cell increase failed to produce any clinical benefit – there was no difference seen in the primary endpoints of rates of death or opportunistic diseases – and IL-2 use was associated with a significant increase in grade 4 clinical events (HR=1.23 p=.003) such as local reactions to the administration of IL-2 (HR=1.92, p=.01) and vascular reactions, primarily deep venous thrombosis (HR=2.81, p<0.001).

SILCAAT, another IL-2 trial, evaluated 1695 subjects who were randomized to receive ART alone or ART plus IL-2 when their CD4+ counts were between 50 and 300 cells/mm³. (Levy Y, et al. Abst. 90bLB) Median follow-up was more than 7 years, and over that time the CD4+ count in IL-2 treated patients was on average 59 cells/mm³ higher than those who received only ART (p<.001). Unfortunately, just as in ESPRIT, there were no significant difference regarding the occurrence of clinical endpoints.

The lack of clinical benefit seen in both ESPRIT and SILCAAT may be due to a variety of factors, including that the expanded CD4+ cells on IL-2 were qualitatively different and not as effective immunologically as normal CD4+ cells. It was also postulated that there may be harmful effects of IL-2 that are not mediated as local reactions to the administration of IL-2 (HR=1.92, p=.01) and vascular reactions, primarily deep venous thrombosis (HR=2.81, p<0.001).
Switching ARV Therapies in Virologically Suppressed Patients

Switching individual ARV agents in virologically suppressed patients represents a potential way of reducing risks associated with ART or managing specific toxicities once they have arisen. Many physicians and patients prefer ‘pro-active’ switching, anticipating a problem or managing a relative risk by switching before a problem arises.

Switching Raltegravir for Lopinavir/r

The SWITCHMRK 1 & 2 studies evaluated individuals receiving successful (screening HIV RNA <50 copies/mL; stable regimen >3 months) LPV/r plus at least 2 NRTIs and randomized them 1:1 to continue LPV/r BID (n=174 in SWITCHMRK 1 and n= 178 in SWITCHMRK 2) or replace LPV/r with RAL 400 mg BID (n=174 & 176, respectively). (Eron J, et al. Abst. 70aLB) Importantly, the studies did not exclude individuals with a history of virologic failure, and, indeed, there was no limit to the number of prior ART regimens. The primary endpoints of the studies were the mean percent change in lipids at week 12 and the proportions of subjects maintaining a HIV RNA <50 copies/mL at week 24 by non-completer equals failure (NC=F) analysis to establish non-inferiority with a 12% margin. Participants were well matched for baseline characteristics, with mean baseline CD4+ count of 471-508 cells/mm³ across arms and only 16.7-18.5% having been on LPV/r <1 year. Subjects had received a median 5-6 prior antiretroviral (ARV) agents over a median of 3.3-4.6 years. The most common concurrent NRTIs were TDF, ABC or ZDV plus 3TC or FTC, with <10% or subjects in each arm receiving 3 or more concurrent agents.

At 24 weeks, the proportion of subjects with an HIV RNA <50 copies/mL (NC=F) in SWITCHMRK 1 were 87% and 81% for the LPV/r and RAL arms, respectively, with a mean difference of 6.6% (95% CI -14.4, 1.2) indicating virologic non-inferiority was not proven. Similarly, in SWITCHMRK 2, the proportion with an HIV RNA <50 copies/mL at week 24 was 94% and 88% for LPV/r and RAL, respectively, with a mean difference of -5.8% (95% CI -12.2, 0.2). A combined analysis across the studies showed 94.4% of LPV/r recipients were responders at week 24 and 89.6% of RAL recipients were responders (mean difference -4.8% 95% CI -9.1, -0.7), which fails to establish RAL non-inferiority. Rebound was noted to be more common among male subjects. Changes in CD4+ were similar across arms in both studies. The proportion of subjects defined as confirmed virological failures (samples taken at least 1 week apart) by cutoffs of <50 and <400 copies/mL is shown in Table 2.

Based on a post-hoc analysis, 27 of 32 (84%) of subjects in the RAL arms with viral rebound were not on their first ART regimen with 18/27 (66%) known to have a history of virological failure on prior therapy.

No safety issues were raised by the study, and, in fact, lipids improved substantially following the switch to RAL. In SWITCHMRK 1, following the switch to RAL, total cholesterol declined 13% (vs. 1% increase in LPV/r arm, p<0.001), non-HDL cholesterol declined 15% (vs. 2% increase in LPV/r arm, p<0.001) and triglycerides declined 41% (vs. 4% increase in LPV/r arm, p<0.001) Similar results were seen in SWITCHMRK 2. Overall, the results from these studies indicate some risk and some benefits to switching from LPV/r to RAL. While both arms saw a high level of treatment success through week 24, there was an excess of viral rebound in the RAL arm. Risk of viral rebound was predominately seen in persons with a history of prior ART exposure, particularly, prior ART failure, suggesting the hypothesis that RAL was not always adequately supported by the weakened NRTI background. Studies in treatment naïve individuals such as the STARTMRK studies indicate high levels of durable efficacy are observed in individuals with 2 fully active NRTIs plus RAL. (Lennox J, et al. 48th ICAAC/46th IDSA, Abst. H-896a)

Switching Raltegravir for Enfuvirtide

In the French ANRS 138 (“EASIER”) study, 170 triple-class resistant subjects with an HIV RNA <400 copies/mL for at least 3 months on an ENF-based regimen were randomized to continue ENF 90 mg BID or replace ENF with RAL 400 mg BID. (De Castro N, et al. Abst. 572) Subjects were well matched at baseline with a median CD4+ count of 393 cells/mm³ from a median nadir of 44 cells/mm³, and had received a median 2.3 years ENF therapy. Through week 24, confirmed viral rebound to >400 copies/mL by ITT analysis was observed in 1/85 (1.2%) ENF recipients and 1/84 (1.2%) RAL recipients (mean difference 0.01%, 95% CI -4.5, +4.5) indicating non-inferiority for the RAL replacement approach. No difference

Table 2. Virologic Failures in SWITCHMRK Studies

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<th>SWITCHMRK 1</th>
<th>SWITCHMRK 2</th>
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<tr>
<td></td>
<td>RAL</td>
<td>LPV/r</td>
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<tr>
<td>&gt;400 copies/mL</td>
<td>3</td>
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<td>&gt;50 copies/mL</td>
<td>13</td>
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in safety events was reported between the two arms. These data contrast with the SWITCHMRK studies and suggest that RAL is a viable replacement agent for other drugs when an appropriately active a background regimen is present.

**Switching to Tenofovir/Emtricitabine or Abacavir/Lamivudine**

The Australian STEAL study compared switching from other NRTI combinations to either TDF/FTC or ABC/3TC, each as a fixed dose formulation, in 360 HLA-B*5701 negative individuals with an HIV RNA <50 copies/mL. (Cooper D, et al. Abst. 576) Approximately 30% of subjects were receiving TDF-based and 20% ABC-based regimens at baseline. The mean CD4+ count was 612 cells/mm³, eGFR 98mL/min/1.73m², limb fat mass 5.5kg and hip t score -0.49. Significantly more ABC/3TC recipients were smokers at baseline (40% vs 29%).

A confirmed viral rebound to >400 copies/mL was observed in 3.9% of TDF/FTC and 5.6% of ABC/3TC recipients (mean difference 1.7%, 95% CI -2.8, 6.1%) indicating non-inferiority to the two approaches. While no new AIDS defining events were observed, serious non-AIDS events were more common with ABC/3TC (4.8%) than TDF/FTC (1.5%) (p=0.022). Specifically, ischemic cardiovascular events (7/179 with ABC/3TC vs. 1/178 with TDF/FTC, p=0.067) were more common on ABC/3TC. Given the differences in baseline cigarette use the significance of this observation is unclear. While new lipid lowering therapy was more commonly initiated in ABC/3TC recipients (9.6% vs 3.7%, respectively p=0.006), declines in hip t score were observed with TDF/FTC (mean change -0.07) compared with an increase in hip t score with ABC/3TC (+0.09) (p<0.0001). The data are supportive of both fixed dose formulations from a viral efficacy standpoint, but raise differences between the agents regarding safety events.

**Antiretroviral Therapies and Cardiovascular Risk**

**DAD Study: Abacavir and Certain PIs Increase CV Risk**

The contribution of ART and specific ARV agents to myocardial infarction (MI) continues to be the subject of intensive investigation. The DAD study has previously described associations with cumulative years of exposure to combination ART, cumulative exposure to protease inhibitors (PIs) and current or recent exposure to abacavir (ABC) and didanosine (ddI). (DAD Study Group. Lancet. 2008;371:1417-26) Support for the association of ABC with MI risk was provided by analysis of the SMART study, which also suggested abacavir recipients showed somewhat higher inflammatory marker (hsCRP, IL-6) levels. (Lundgren J, et al. AIDS 2008;22:F17-F24)

These observations were not supported by GlaxoSmithKline’s safety database or analysis of the prospective HEAT study. (Caturell A, et al. 17th IAC, Abst. WEAB0106)

As more events accumulate in the DAD cohorts, the ability to assess associations with specific agents increases. The DAD study was updated for the 16th CROI, and this iteration included 580 MIs evaluated across 178,835 person years of follow-up. (Lundgren J, et al. Abst. 44LB) Individual agents associated with an increased relative risk (relative risk [RR], 95% CI) of MI by cumulative years of exposure were ABC (RR 1.07, 1.01-1.14), indinavir (IDV) +/- RTV (RR 1.12, 1.07-1.18), and LPV/r (RR 1.13, 1.05-1.21). The association with these PIs was partially corrected for by adjusting for lipids suggesting PI agents with lesser effects on lipids should be preferred pending more data. Other ARV agents, including TDF (RR1.05, 0.92-1.19), nelfinavir (NFV), SQV, nevirapine (NVP) and Efavirenz (EFV), were not associated with increased risk. Assessment of more recently approved agents such as atazanavir (ATV) and darunavir (DRV) are not yet available. When current or recent use of NRTIs and MI relative risk were considered, ABC (RR 1.68, 1.33-2.13) and ddI (RR 1.41, 1.09-1.82) remained significantly associated with MI, whereas TDF (RR 1.14, 0.85-1.52) was not.

**ANRS CO4: Increased CV Risk with Abacavir**

A case controlled study by the French ANRS group, which included 268 cases of MI within their database matched with 865 MI-free controls for age, sex and care center, also assessed the cardiovascular risk (odds ratio [OR], 95% CI) associated with ABC. (Lang S, et al. Abst. 43LB) It found an association with <1 year or recent ABC use and MI (OR 2.19, 1.19-4.02) but not other NRTIs. Unlike the DAD findings, however, cumulative exposure to ABC was not associated with MI. Cumulative exposure to LPV/r (OR 1.38/year, 1.1-1.74) and fosamprenavir (FPV) (OR 1.55/year, 1.19-2.02) were also significantly associated with MI risk.

**ACTG 5001: No CV Risk with Abacavir**

In contrast to the DAD and French studies, ACTG A5001, which combined 5 prospective ACTG studies with 27 MI events, and 63 severe CVD events across 10,187 patient years of follow-up, found no association between recent ABC use and either event type. (Benson C, et al. Abst. 721) The relatively low event numbers and smaller number of
patient years of follow up in this study leave wide confidence intervals around the conclusions and so do not rule out a possible modest effect.

**Potential Mechanisms of CV Risk**

Given the relative reproducibility of the association between recent ABC use and MI a mechanism is being sought. In the Womens’ Interagency HIV study and the Multicenter AIDS Cohort Study no associations with ABC and elevated levels of inflammatory markers hsCRP, IL-6 or the clotting factor d-dimer were found. (Palella F, et al. Abst. 150LB) These observations were supported by the prospective HEAT study comparing ABC/3TC with TDF/FTC, each with LPV/r, which found no differences in hsCRP, IL-6 and soluble vascular cell adhesion molecule-1 (sVCAM-1), a marker of endothelial dysfunction. (McComsey G, et al. Abst. 732) However, other groups noted platelet hyper-reactivity in ABC recipients relative to matched individuals receiving alternative NRTIs and that vascular reactivity as measured by brachial artery reactivity was more impaired in HIV RNA suppressed ABC recipients than in persons on alternative NRTIs (Satchell C, et al. Abst. 151LB; Hsue P, et al. Abst. 723) On a more positive side, in the ACTG 5206 study, the addition of TDF to an on-going suppressive regimen resulted in significant declines in pro-atherogenic lipids including a median 39 mg/dl decline in total cholesterol, similar to observations of use of this agent reported as monotherapy in healthy volunteers (Tungsiripat M, et al. Abst. 714; Randell P, et al. 48th ICAAC/46th IDSA, Abst. H-2307)

**Antiretroviral Therapies and Renal Risk**

While lipid and MI risk data with TDF regimens are reassuring, investigators continue to evaluate the safety of this agent with regard to potential adverse renal effects. In 115 individuals on TDF for a median 35 months, 19 were noted to develop some renal tubular dysfunction. A genetic factor, a GG allele in the ABCC2 gene encoding for a renal transporter, was associated with a 5-fold increased risk of tubular dysfunction. Other independent risk factors were age and weight. (Rodriguez Novoa S, et al. Abst. 37)

In the prospective HEAT study, proximal renal tubular events were rare – 5 cases in 395 individuals treated with TDF over 96 weeks – and only seen in the TDF/FTC arm. (Fine D, et al. Abst. 744) Overall, however, as shown in figure 2, renal function modestly improved in both groups.

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**Factors Affecting Antiretroviral Efficacy**

**ARTEMIS Study:**

*DRV/r vs. LPV/r in ARV-naïve Patients*

Darunavir (800 mg) boosted with ritonavir (100 mg) (DRV/r 800/100) once daily (QD) was approved in both Europe and the US for the treatment of HIV-1 infection in treatment-naïve adult patients following the report of the ARTEMIS study 48-week results. (Ortiz R, et al. AIDS 2008;22:1389–97) This study is an ongoing, randomized, controlled, phase 3 trial evaluating the efficacy and safety of DRV/r 800/100 QD versus LPV/r given either QD or BID in treatment-naïve, HIV-1-infected patients. At 96 weeks, 79% of DRV/r compared with 71% of LPV/r patients achieved an HIV RNA <50 copies/mL in an intent-to-treat/time-to-loss of virologic response (ITT-TLOVR) analysis (p value for superiority = 0.012). (Mills A, et al. 48th ICAAC/46th IDSA, Abst. H-1250c)

In a re-analysis of the ARTEMIS data presented at this conference, the study authors investigated factors that may have influenced the virologic responses between both treatment groups such as adherence, and other prognostic covariates including age, sex, race, region, adherence, and baseline HIV RNA and CD4+ cell count. (Nelson M, et al. Abst. 575)

Adherence was measured by the Modified Medication Adherence Self-Report Inventory (M-MASRI) questionnaire which assessed adherence by percentages of doses taken from Week 0 to Week 96. The average adherence from Week 4 to Week 96 was used to assess overall adherence. A mean adherence >95% was considered “adherent” and a mean adherence of <95% was considered “sub-optimally...
adherent”. As shown in figure 3, the difference in response between patients adherent and sub-optimally adherent in the DRV/r arm was very small and not statistically significant. To the contrary, patients receiving LPV/r who had sub-optimal adherence were significantly more likely to fail therapy.

Figure 3. Proportion of patients with VL <50 copies/mL by average adherence.

Other analyses were performed to assess whether adherence and other factors in the patient's baseline characteristics may have affected the differences in response that were observed. Using a multivariate analysis, DRV/r superiority was maintained after adjusting for adherence, viral load, CD4+ count, age and race. Further, these analyses found that efficacy benefits seen in the DRV/r arm were driven primarily by virologic endpoints, and they were not primarily caused by differences in discontinuations due to adverse events or other reasons.

It is very possible that these differences can be explained on the basis of drug levels. These data correlate with PK data presented on these PIs by others (Boffito M, et al. EACS 2007, LBPS7/420; De Jesus E, et al. 47th ICAAC, Abstract 718b) in which for patients lacking adherence, or taking their medications late, the pharmacokinetics of LPV/r were found to be significantly compromised. In those studies, the trough LPV concentrations of patients taking LPV/r twice daily rapidly declined if they were late with a dose and the PK was much worse for patients taking the LPV/r once daily. On the contrary, DRV/r appears to have much better forgiveness, with trough concentrations several fold above the EC50, and this may be expected to protect the patients with adequate therapeutic levels for longer periods of times should they be late with or miss a dose.

BENCHMRK 96-week data: Raltegravir in Heavily-Experienced Patients

BENCHMRK 1 and 2 are ongoing phase III, double blinded studies that enrolled triple-class resistant patients failing their current ARV therapy. The enrolled patients were randomized 2:1 to receive either RAL or placebo, both with an optimized background regimen (OBT). Because of similarities in study design and patient baseline characteristics the results of these 2 studies are usually combined into a single report. At 96 weeks, RAL plus OBT continued to show a potent, superior, and durable antiretroviral efficacy. At 96 weeks, 57% and 26% of the patients in the RAL and placebo arms, respectively, maintained an HIV RNA <50 copies/mL (p<0.001). (Steigbigel R, et al. Abst. 571b) The immunologic responses remained equally impressive with a mean CD4+ count gain in the RAL + OBT arm of 123 cells/mm³ vs. 49 cells/mm³ in the placebo + OBT arm. There were 25 new virologic failures between weeks 48 and 96, 8 (7.6%) in the placebo and 17 (4.6%) in the RAL arm. The RAL failures were generally associated with mutations at one of three primary residues, Q148, N155, or Y143, in combination with at least one other mutation. It is clear that the data at 96 weeks in these studies continues to demonstrate the superiority of RAL over placebo. The virologic responses and tolerability initially observed at 48 weeks are sustained, with minimal indication of patients losing virologic suppression or discontinuing therapy.

STARTMRK subgroup analysis: Raltegravir vs. Efavirenz in ARV-naïve Patients

The STARTMRK trial is an ongoing, randomized, placebo-controlled trial, in which RAL 400 mg BID is compared to EFV 600 mg QD, both in combination with TDF/FTC, in ARV-naïve patients. The results at 48 weeks demonstrated the non-inferiority of RAL to EFV in terms of patients achieving virologic suppression, while the RAL arm was also associated with fewer adverse events and greater CD4+ cell count increase. (Lennox J, et al. 48th ICAAC/46th IDSA, Abst. H-896a) In this analysis, none of these subgroups
were identified to be associated with suboptimal virologic or immunologic responses. The results of this study are important because as previously stated, RAL, as the first member of the new integrase-class agents approved for the treatment of HIV infected patients, needs to demonstrate similarities in virologic and immunologic responses across a variety of patient groups.

**Predictors of IRIS and Mortality in ART-Treated Patients with Acute Opportunistic Infections**

One of the major concerns of initiating antiretroviral therapy in patients with advanced HIV disease is the occurrence of the immune reconstitution inflammatory syndrome (IRIS). IRIS represents an increase in the inflammatory response to either a previously-diagnosed or subclinical OI, with symptoms ranging from mild (low-grade fever, adenopathy) to life-threatening (increase in intracranial edema with toxoplasmosis, or pulmonary infiltrates due to PCP). Retrospective studies have estimated an incidence of IRIS from 10-40%, depending on the population evaluated. ACTG 5164 examined early vs. delayed ART in patients presenting with acute HIV-related OIs, exclusive of tuberculosis; study results presented at last year’s CROI found a diminished rate of progression to a new AIDS defining event or death among those receiving early ART. (Zolopa A, et al. 15th CROI, Abst. 142) Presented here is an analysis of risk factors for both IRIS and mortality among these patients in the study. (Grant P, et al. Abst. 775) For the purposes of this protocol, IRIS was defined as (1) evidence of an increase in CD4+ cell count and/or a decrease in the HIV-1 viral load in response to starting ART with (2) symptoms that are consistent with an infectious/inflammatory condition and temporally related to initiation of ART but cannot be explained by a newly acquired infection, the expected clinical course of a previously recognized infectious agent, or the side effects of ART itself.

Two-hundred eighty-two patients were enrolled, out of whom 262 started ART; they had a median CD4+ count of 29 cells/mm³ and HIV RNA of 5.1 log_{10} copies/mL. *Pneumocystis jiroveci* pneumonia (PCP) was the most common OI, occurring in 64% of patients; other OIs were bacterial infection (15%), cryptococcus (15%), mycobacterial infection (6%), toxoplasmosis (5%), CMV (4%), and histoplasmosis (4%).

IRIS was diagnosed in 20 (7.6%) of patients (8 receiving early ART, 12 delayed); it occurred after a median of 33 days on ART. No difference in the incidence of IRIS was noted among those who did and did not receive corticosteroids, although no case of IRIS was diagnosed among those on current corticosteroids. Occurrence of IRIS was associated with a greater HIV RNA decline at week 4, but not changes in the absolute CD4+ count; the only baseline characteristic associated with greater risk of IRIS was the diagnosis of a fungal infection. Twenty-three out of 282 patients died during the study. In multivariate analysis, entry mycobacterial infection (HR=4.6, p<0.01), hospitalization (HR=3.2, p<0.01), and low CD4+ count (HR=1.2 for each 10 cells/mm³ decrement, p=0.04) predicted mortality.

The authors concluded that the incidence of IRIS is likely overestimated in retrospective studies, noting that the incidence described here of 7.6% is similar to that reported in a prospective study from South Africa. In addition, it is not possible to predict who will develop IRIS solely from baseline characteristics. The finding that a lower CD4+ cell count is still predictive of increased risk of mortality even among these patients with highly advanced HIV disease demonstrates the critical role of starting ART those with most severe immunosuppression. Fortunately, early ART does not appear to increase the risk of IRIS, at least among those with non-TB OIs.

**Pharmacokinetic Issues**

**Potential Replacements for Ritonavir: GS-9350 and SPI-452**

The use of RTV, a potent CYP 3A inhibitor, to boost drug levels of PIs has been critical to the success of this drug class. Currently, every PI recommended by the DHHS and IAS-USA guidelines is RTV-boosted. (DHHS Guidelines, November 3, 2008 http://aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf; Hammer S, et al. JAMA 2008;300(5):555-570) Despite the successes of the PI class, there are lingering concerns with the long term tolerability and toxicity of RTV, especially RTVs gastrointestinal tolerance and its adverse affect on lipid levels. There were two studies at CROI that explored the possibility of alternative boosting agents.

The first study concerned a novel boosting agent, GS-9350, which in preclinical studies appeared to be a potent inhibitor of CYP 3A, without any HIV activity. (Kearney B, et al. Abst. 40) This Phase I study was a dose escalation strategy for 12 healthy volunteers testing 50, 100 and 200 mg of GS-9350 compared to 3 who got RTV or a placebo. This study extended over 14 days and used a marker of CYP 3A...
inhibition, Midazolam (MDZ), as a probe to determine the extent of inhibition. There was a non-linear relationship of drug concentration with the highest dose of GS-9350 (200mg) being similar to 200 mg of RTV (both with 95% inhibition of MDZ metabolism by CYP 3A), however, the 100 mg dose of GS-9350 also demonstrated 92% inhibition.

All doses were well tolerated and there were no drug-related Grade 3/4 laboratory abnormalities. There were no changes or differences seen in lipids across all subjects for 14 days.

Based on this trial, a pilot study to evaluate a fixed-dose tablet containing GS-9350, Elvitegravir (EVG) 150 mg, and TDF 300 mg and FTC 200 mg was performed. Forty-four healthy volunteers were recruited and given EVG/TDF/FTC plus either 100 mg or 150 mg of GS-9350 in a fixed dose tablet and the concentration gradients of EVG were measured compared to RTV boosted EVG. The two different doses produced levels that were considered therapeutic and the 150 mg dose maintained levels that were 11 fold greater than IC$_{50}$ for EVG, without any changes in levels of TDF or FTC. These treatments were well tolerated with two grade 3 adverse events, ALT elevation (GS9350 100 mg FDC), one subject with appendicitis and no other grade 3 or 4 lab abnormalities.

The second study evaluated SPI-452. (Guttendorf R, et al. Abst. 41) Study 0452-001 had 67 subjects who took two different PI agents, either ATV 300 mg or DRV 600 mg, as a single dose to determine what boosted levels of these drugs could be achieved. They were followed for an additional seven days to allow the drug to washout and then were given increasing dosages of a once daily SPI-452 (20 mg, 50 mg, or 200 mg or placebo) for fifteen days. The PI agents, Darunavir 600 mg and Atazanavir 300 mg were then given on the fifteenth day, as well as a placebo. On day 16, they received only the PI agents and the placebo without any of the previously used booster.

Results showed that SPI-452 was well absorbed and reached a steady state level within 14 days. There was a pronounced boosting effect on the peak concentration of both DRV (37 fold increase) and ATV (13 fold). This boosting effect persisted through day 16, when SPI-452 had been discontinued the day prior. Headache, nausea/vomiting and diarrhea were the most common adverse events and there were no signals of any safety issues during this 15 day study. One of the key remaining hurdles for this compound will be formulation, as it is currently in a liquid form.

**Raltegravir and atazanavir PK interactions**

Because of their unique metabolic pathways, the combination of ATV and RAL has been thought to render beneficial pharmacokinetic properties. ATV is a direct inhibitor of uridine diphosphate-glucuronosyl transferase (UGT) 1A1, which is the major mechanism for clearance of RAL.

To demonstrate the PK of these agents when they are concomitantly administered, a 2-way PK study was conducted in healthy volunteers using ATV 300mg and RAL 400mg, with both given BID. (Zhu L, et al. Abst. 696) Nineteen of the 22 subjects enrolled completed the study. They received RAL 400mg BID for the first 5 days, ATV 300mg BID from day 6 to 12, and a combination of both for 2 weeks thereafter. Trough concentrations were measured at regular intervals, as well as routine lab data and serial ECGs.

Co-administration of ATV 300mg and RAL 400mg BID decreased ATV AUC and Cmin relatively to ATV BID alone; but all ATV Cmin were >10 times the concentrations needed to suppress wild-type virus. RAL concentrations, on the other hand, increased to a similar extent observed when RAL is co-administered with ATV/r 300/100 mg.

No ECG changes were noted during the RAL alone dose period. But the mean QRS increased from ATV baseline (mean 88, range 72-101 ms) to day 12 (mean of 11 ms, range 2-25ms). No QRS interval was >120ms during ATV administration. There were also mild increases in the PR interval during the same period of time. The significance of these ECG changes is unclear and warrants further investigation. Hyperbilirubinemia was comparable to historical data with boosted ATV. No other major adverse events were observed.

This combination of ATV + RAL potentially provides a nucleoside-sparing and RTV-sparing regimen. Given the known potency and safety profiles of both drugs, the ATV + RAL combination is expected to show good clinical efficacy while offering a good safety and tolerability profile, which is needed for long-term treatment of HIV infection. A pilot study looking at this treatment strategy in naive patient is currently underway.