

Synopsis: Report Back from CROI

The *Report Back From CROI*, the Community Consortium's annual recap of highlights from the Conference on Retroviruses and Opportunistic Infections, took place on March 9, 2005, at UCSF Mission Bay's Genentech Hall auditorium. Donald Abrams, MD, Chair of the Consortium, moderated and the panel of speakers included Stephen Follansbee, MD, Director of Kaiser San Francisco HIV Services, Harry Lampiris, MD, Associate Professor of Clinical Medicine, SFVA/UCSF, and Steven Deeks, MD, Associate Professor of Medicine at the UCSF Positive Health Program at San Francisco General Hospital. Over 100 health care professionals and other community members were in attendance. Dr. Abrams greeted the audience and introduced the panel members, then explained the round-robin format of the evening's program. He asked the speakers to begin their presentations by giving their overall impressions of the conference.

FOLLANSBEE (one)

Dr. Follansbee opened by saying he thought this year's conference dealt less with overall strategies of HIV treatment and addressed more specific management issues. He felt the most exciting areas were again, as last year, virology and the pathogenesis of immune deficiency with HIV infection. As usual, he said, it was the year's most exciting and informative conference.

Therapeutic Drug Monitoring

His first topic of discussion for the evening was the utility of therapeutic drug monitoring (TDM). He asked for a show of hands from audience members who were currently checking drug levels on their patients, and a fair amount indicated that they were. It has been done in a few patients at Kaiser, he said, as they were switched from amprenavir to fosamprenavir, and he has become more interested in the concept. It's not clear, though, exactly what place there is in the clinic for drug level monitoring, as several presentations at CROI made apparent.

Collier et al. presented results of ACTG 5143, which was stopped early because of pharmacokinetic (PK) sub-study data. This randomized study, which enrolled protease inhibitor (PI) -experienced patients who had failed antiretroviral therapy, was designed to determine whether lopinavir (LPV) 400 mg/ritonavir (r) 100 mg + fosamprenavir (FPV) 700 mg twice daily (double PI) leads to superior HIV-1 RNA response compared with LPV/r or FPV + r (single PI). Background therapy was tenofovir (TDF) and 1 or 2 "optimal" NRTIs determined by virtual phenotype. The PK interim analysis results, published earlier this year by Kashuba, showed inadequate minimum concentrations of LPV and APV in the double PI arm, significantly lower than in the single PI arms. Enrollment was therefore stopped at 56 of a planned 216 subjects, study duration was shortened from 48 to 24 weeks, and subjects on the double PI arm were made to stop LPV/r or FPV (based on previous PI experience). However, virologic responses (1 log₁₀ drop in HIV-1 RNA or greater, or <50 copies/ml) at week 24 were 75% with double (n = 28) and 61% with single (n = 28) PI by intent-to-treat (ITT) analysis; as-treated (AT) results were 100% with double (n = 12) and 64% with single (n = 25) PI. CD4⁺ cells increased a median of 81 and 41/mm³ (ITT) and 114 and 43 /mm³ (AT) in double and

single PI groups, respectively. By ITT analyses, the differences in viral and CD4⁺ cell responses did not reach significance, but in the AT analyses trends favored the double PI group. Comparison was limited by early termination and the resulting small sample size, but certainly the double PI group did not suffer virologic harm. While it was the correct decision to stop enrollment early based on the PK information, Dr. Follansbee said, this case shows us that our knowledge of how to make use of such information remains limited.

There was good news from a second presentation on TDM, from Paul Pham et al., who evaluated the steady-state PK of amprenavir (APV) + Kaletra (LPV/r), with or without efavirenz (EFV). The findings are particularly relevant, Dr. Follansbee noted, “for those of us in salvage mode.” The three drugs are often used in salvage regimens because their resistance profiles do not overlap; however, because they are all metabolized via cytochrome P450 3A4, there has been concern about potential drug interactions. This study found, in a comparison of individuals taking APV + LPV/r (*n* = 12) versus those taking APV + LPV/r + EFV (*n* = 7), that efavirenz did not affect the PK profile of either PI.

The 2NN study (F van Leth et al.) looked at predictors of virologic success based on trough levels of nevirapine (NVP) vs. EFV. NVP and EFV concentrations were assessed at day 3 and week 1, 2, 4, 12, 24, and 48. Virologic failure was defined as never a plasma HIV-1 viral load <50 copies/mL or a rebound to 2 consecutive plasma viral load readings of >50 copies/mL. In looking at minimum concentrations (*C*_{min}) of NVP, the study team found no cut-off value below which a significant increased risk of virologic failure occurred. The cut-off value for an increased risk of failure with EFV was a *C*_{min} <1.1 mg/L; however, there was a significant number of patients with a *C*_{min} <1.1 mg/L who still had virologic success at 48 weeks. Thus, asked Dr. Follansbee, should we boost NNRTI levels based on a low *C*_{min} value, when there is no clear evidence from this large study that the value predicts failure?

Mentre et al. conducted a TDM study (COPHAR2-ANRS 111) in PI-naive patients who began treatment with RTV-boosted indinavir (*n* = 42), Kaletra (*n* = 38) or nelfinavir BID (*n* = 35). This was an open, non-comparative multicenter prospective trial, with repeated therapeutic drug monitoring at weeks 2, 8 or 16, 24, and 48. PI dose adjustments were made at week 24 if trough levels were outside the therapeutic range, which was quite broad for each PI. Adjustments were made in increments of 1 pill per day. The high rate of NFV trough levels below the therapeutic range led the study team to amend the protocol to provide an RTV boost for those patients with low concentrations. Regimen failure was defined by either 2 consecutive viral loads ≥ 200 copies/mL between weeks 16 and 48, or a PI-related adverse event grade 3 or 4 or a grade 2 dose-dependent event like diarrhoea or renal colic. Patients without early adverse events were defined as assessable if they had at least the week 16 virologic assessment. There were 30 IDV/r patients, 32 Kaletra patients and 32 NFV patients that were assessable. Success rates for the strategy (i.e., virologic success and no PI-related adverse event) were 70%, 69% and 44%, respectively, by intent-to-treat analysis; on-treatment analysis showed rates of 87%, 81% and 56%, respectively. There was a trend toward improved outcome using *C*_{min}

measurement, but there was not sufficient evidence, in Dr. Follansbee's opinion, of clinical utility.

A team at Johns Hopkins (Nettles et al.) looked at frequent PK sampling as a means of determining intra-individual pharmacokinetic variability. In 10 patients with undetectable plasma HIV RNA (< 50 copies/mL) who were stable on their current regimen for at least 3 months, they measured PI and NNRTI concentrations, along with HIV RNA levels, 3 times a week for 3-4 months. Plasma was collected at the same time of day every Monday, Wednesday and Friday, and patients were instructed to take their medications at the same time every day. Viral loads were measured in order to define the frequency of virologic "blips" (isolated plasma HIV RNA \geq 50 copies/mL). Pharmacokinetic variance was expressed as intra-individual % coefficient of variation (ICV), or standard deviation divided by mean drug level over the study period. Of 713 samples, "blips" were seen in 26 (3.6%); they did not, however, correspond with low drug concentrations. Median ICV% was unexpectedly high: 43% among PIs, 24% among NNRTIs. This wide variability indicates, again, that TDM may be of limited clinical utility.

DEEKS (one)

Dr. Follansbee ended his opening remarks at this point, and Dr. Deeks came to the podium. He loves the Retrovirus Meeting, he said, because of the wealth of data provided there. This year he felt that there was a focus, more so than in the past, on international issues; there was also a good deal of generated in the area of basic virology. Clinical trials were not as well represented this year as in the past, nor were immunology, pathogenesis and other basic science. It was nonetheless a successful conference, he thought, despite the unfortunate dominance of the "New York Case" (an HIV-positive individual with multidrug-resistant, dual tropic HIV-1 and rapid progression to AIDS) as a discussion topic.

Taking a brief moment to underscore the lack of consensus regarding TDM, he mentioned that Charles Flexner - part of the Johns Hopkins study team, and a pharmacologist whose opinion he respects - had looked at variability in specific individuals on study. His conclusion was that, outside of a clinical trial setting where multiple samples are drawn over time in controlled circumstances, it does not make sense to make therapy changes based on TDM results. Also stemming from this trial was Bob Siliciano's finding that "blips" between 50 and 200 copies/mL do not represent viral evolution; they are instead, in large degree, the result of assay variability. This particular information was recently published in JAMA.

The great unanswered question of the conference, said Dr. Deeks, was, "Is X4 virus bad for you?" As we are in the process of using CCR5 inhibitors, and developing more of them, we will be seeing more and more CXCR4 virus. What will the consequences be? He was surprised not to see this compelling and controversial issue receive more attention as, he warned, clinicians will be dealing with it in the coming months.

Tipranavir vs. TMC 114

Another current question concerns the construction of PI-containing salvage regimens: Should clinicians use tipranavir or wait – probably not too long – for TMC 114 to become available? Tipranavir is expected receive approval from the FDA in the next few months [granted June 22], and results from the late phase III RESIST 1 and 2 studies were presented by Cooper et al. The RESIST studies are multicenter, open-label trials in treatment-experienced patients randomized to a standard-of-care regimen containing either a boosted comparator protease inhibitor (PI) (CPI/r) or tipranavir/ritonavir (TPV/r). The objective of this analysis was to compare the efficacy of TPV/r and lopinavir/ritonavir (LPV/r), and to assess the role of additional active drugs in the optimized background regimen (OBR). Qualifying patients were triple class-experienced (having taken at least 2 PI-based regimens) with viral loads over 1000 copies/mL; they were required to have at least 1 primary PI mutation but no more than 2 at amino acids 33, 82, 84 and 90. An optimized CPI/r regimen was selected by genotype prior to randomization. Patients then received either TPV/r (500 mg/200 mg bid) or the preselected CPI/r plus the OBR. Quite promising data from RESIST 1, favoring TPV/r, were presented at ICAAC; however, the study team elected to further examine TPV/r in a specific comparison with LPV/r, the current standard salvage PI. Thus, using the patients on the TPV/r arm who had selected Kaletra as their CPI/r before randomization, the team compared treatment responses to TPV/r vs. LPV/r and evaluated the effect of active agents (those with predicted antiretroviral sensitivity based on genotype) in the OBR. Treatment response was defined as a confirmed ≥ 1 log₁₀ decrease in viral load from baseline. At week 24, treatment response by intent-to-treat analysis for TPV/r (39.6%) was significantly higher than for LPV/r (21.4%). Comparing against LPV/r patients who were actually naive to LPV/r at baseline, the difference was not statistically significant, though there was a trend favoring TPV/r. We can conclude that TPV/r is probably a better drug in terms of efficacy, said Dr. Deeks, but we should keep in mind the potentially serious side effects associated with TPV/r. TPV/r patients on this study experienced grade 3 and 4 elevations in ALT, cholesterol and triglycerides at a significantly higher rate than did LPV/r patients.

About 25% of patients overall took T20, and the RESIST team looked at its effect on treatment response. To their surprise, 63.5% of these subjects who were T20-naive achieved a treatment response, an impressive result for a salvage study, especially compared to the 36.7% rate of response in T20-naive subjects who did *not* take T20. In T20-experienced patients those levels were 30.6% and 7.3%, respectively.

The drug that received the most attention at the conference was the protease inhibitor TMC114, currently entering phase III studies. It should be available via expanded access within the next year or two. The primary issue with TMC114, Dr. Deeks stated, is whether it is a better drug than tipranavir, and thus whether clinicians should bypass TPV, if possible, and wait for TMC114. Another question, one that cannot yet be answered, is whether TPV failure will weaken or perhaps nullify the effect of TMC114. Katlama et al. described an efficacy study of TMC114/r in 497 triple-class-experienced patients who were on a PI-containing regimen and had at least one primary PI mutation. These heavily pre-treated patients were randomized to 1 of 4 TMC114/r dose regimens ($n = 397$) or a

comparator PI ($n = 100$), with an optimized background regimen with or without T20. Dose studies were 400/100 QD, 800/100 QD, 400/100 BID and 600/100 BID. A dose response comparison at week 24 found that the proportions of patients with viral loads <50 copies/mL were 30%, 31%, 38% and 47%, respectively; all responses were highly significant compared to the comparator PI arm, at 9%. The 600/100 BID dose will go forward in the phase III trials.

As with tipranivir, there is a synergistic relationship between TMC 114/r and T20, as the team found in an analysis of the 600/100 BID subset. Of the T20-naive patients who were coadministered T20, 67% achieved a viral load <50 copies/mL at week 24, compared with 16% on T20 alone and 37% on TMC 114/r alone. Nearly half of the patients (with or without T20) with 3 or more primary mutations likewise had viral loads below detectability. Even in those who were resistant to all the background antiretroviral agents, 31% had this optimal result.

All in all, Dr. Deeks concluded, the data are impressive for both tipranivir and TMC 114. Less ritonavir is required with TMC 114, so the risk of side effects may be slightly improved. In patients for whom a regimen change is more urgent, there is no reason to wait for TMC 114 to become available. In patients that can wait, it is certainly feasible to wait for more complete data on TMC 114.

LAMPIRIS (one)

There is an overwhelming amount of information at CROI, said Dr. Lampiris, and tonight's panelists chose specific areas of focus, inevitably overlooking a number of the conference's important presentations and discussions. He encouraged those present to investigate the many abstracts and sessions available on the Website, including information on mother-to-child transmission and nevirapine use, the 3 x 5 Initiative, and the complications associated with introducing antiretroviral therapy to the third world. There is much to learn about treatments and treatment strategies, he said, as their use increases in these new populations.

He shared a cheering presentation, from Walensky et al. at the Harvard School of Public Health, entitled, "2 Million Years of Lives Saved, US, 1989-2003." It concerned a study of all people in the United States who had access to treatment and the dramatic changes in survival gain associated with 6 successive and additive phases of therapeutic advancement: PCP prophylaxis in 1989, MAC prophylaxis in 1993, the availability of potent new antiretroviral agents in 1997, more effective combination regimens in 1998, simplified regimens in 2000, and less toxic regimens in 2003. The estimated total gain, around 2 million years of US lives saved, is remarkable. Just as remarkably, the survival benefit per person is quite a bit higher than those conferred by other chronic disease therapies. These findings underscore the importance of effective HIV care and continued research efforts.

Lipoatrophy and Hyperlipidemia

Dr. Lampiris chose to begin his presentation by sharing some new developments in the area of metabolic abnormalities associated with the use of HAART. Looking first at the potential for reversing the fat-diminishing effects of thymidine analogs (t-NRTIs), he described Graeme Moyle's study of virologically suppressed patients with moderate to severe lipoatrophy who switched from t-NRTIs (d4T or AZT) to either tenofovir or abacavir. Gilead's TDF licensing study of several years ago, in which TDF/3TC/EFV was compared to d4T/3TC/EFV, showed that continued use of d4T was associated with significant loss of peripheral fat while, conversely, TDF use correlated with peripheral fat gain. Moyle's 48-week study compared the effects of the TDF and ABC substitutions with regards to limb fat recovery, change in lipids, and virologic control. Subjects' limb fat was measured at regular intervals by DEXA, their visceral and subcutaneous abdominal fat by CT. Viral loads, adverse events, and fasting metabolic parameters were also tracked. At 48 weeks, both arms showed similar and significant gains in limb fat, visceral fat, and subcutaneous abdominal fat. Both groups maintained viral suppression; however, measurements of lipid changes significantly favored TDF, and drug discontinuation because of toxicity was more common with ABC (due to hypersensitivity reactions in 3 of the 6 cases) than with TDF (1 discontinuation). The average increase in body fat after one year was 0.4-0.5 kg (approximately 10% of total fat) and Dr. Lampiris pointed out that this change, while measurable, may not be visible and thus significant to the patient. Whether the improvement continues at the same rate over time, resulting in visible change, is of course yet unanswered.

ACTG 5110 also looked at switching off t-NRTIs to improve lipoatrophy (Murphy et al.), in this case randomizing 101 participants with peripheral fat loss and viral loads below 500 copies/mL to (1) exchange d4T or AZT for abacavir, (2) switch to the NRTI-sparing regimen LPV/r + NVP, or (3) delay changing regimens for 24 weeks. Switching immediately to a nonthymidine analog or to no NRTI at all was associated with significant improvement in various measures of body fat, while retaining the t-NRTI resulted in further fat loss. Of note, fat gains were significantly better in the NRTI-sparing regimen than in the ABC regimen. The subjects did quite well virologically across arms, almost all of them maintaining viral loads less than 200 copies/mL.

Tebas et al. presented a third evaluation of regimen change in the context of lipoatrophy. ACTG 5116 was a metabolic substudy of ACTG 5125 in which 62 participants were randomly assigned either to continue their NNRTI + 2 NRTI regimen or to switch to EFV + LPV/r. Results at 48 weeks showed an increase in limb fat in the NRTI-sparing arm (+562 g) versus a decrease in the NRTI arm (-246 g), as measured by DEXA. Lipid derangements were, predictably, significantly worse in the NRTI-sparing group. There were no significant changes between arms or within arms in fasting glucose and insulin levels, truncal fat or bone density. Though the study was not powered to detect differential effects of specific NRTIs used in the NRTI arm, it does add to the evidence implicating this drug class in the peripheral fat loss that characterizes HIV lipoatrophy.

Moving on to hyperlipidemia, Dr. Lampiris discussed a study of fish oil for triglyceride elevations induced by antiretrovirals (De Truchis et al.). Studies in HIV-negative

populations have indicated that fish oil (Omega-3 polyunsaturated fatty acids) offers coronary protection, perhaps acting as an anticoagulant, inhibiting vitamin K dependent clotting factors; it also has an anti-platelet effect. In addition to these aspirin-like properties, however, fish oil has been shown - again, in HIV-negative subjects - to reduce serum triglyceride levels by approximately 25%. De Truchis et al. presented safety and efficacy data from a prospective, double-blind study of fish oil (Maxepa) in 122 HIV-infected patients on antiretroviral therapy who, after 4 weeks of dietary modification, still had triglyceride levels of 2g/L or higher. Patients received either Maxepa 2 g TID ($n = 60$) or placebo ($n = 62$) for 8 weeks, followed by open-label Maxepa for an additional 8 weeks in both arms. Those with baseline triglyceride levels greater than 10 g/L were not randomized and were given open-label Maxepa. At 8 weeks, the median reduction in triglycerides in the Maxepa arm was 25.5%, compared to a 1% gain in the placebo arm, and triglyceride levels normalized in 22.4% of patients taking Maxepa. No change in total or HDL cholesterol was detected in either arm. There was no difference between groups in the occurrence of adverse events, though Dr. Lampiris pointed out that there is an increased risk of bleeding for patients taking aspirin or Coumadin. Of note, fish oil does not interact with HIV medications, which gives it a distinct advantage over the statins and fenofibrates currently used for hyperlipidemia.

FOLLANSBEE (two)

Renal Complications

Dr. Follansbee returned to the podium with a discussion of HIV, HAART and renal dysfunction, a topic well represented at CROI. Riesler et al., using the MACS data, presented information on the prevalence and predictors of chronic kidney disease (CKD) in HIV-infected men. They looked at 1470 HIV-positive and HIV-negative individuals and determined their glomerular filtration rates (GFR) based on serum creatinine level, age, race, and weight; the GFR in turn allowed CKD staging. The team evaluated the relationships between HIV status, HAART use, and the presence of more advanced CKD stage, controlling for such factors as pre-HAART HIV-1 RNA level, pre-HAART CD4 cell count, hypertension, diabetes mellitus, smoking, and body mass index. Recent use of tenofovir was also considered. HIV-positive men on HAART were more likely to have stage 3-5 CKD compared to HIV-negative men but they did not differ in likelihood of stage 2 CKD. Of HIV-positive men, those using HAART were more likely to have stage 2-5 CKD than those who were HAART-naïve. Pre-HAART viral load and CD4 cell count were not associated with GFR, nor was length of time on HAART. However, use of tenofovir was associated with lower GFR than in non-users.

A second presentation (Steve Becker et al.) reported results from an analysis of rates of renal insufficiency in Glaxo Smith-Kline's CHORUS study. Following 1298 participants on tenofovir prospectively for a median duration of 16 months, the investigators compared identification of renal insufficiency by GFR vs. serum creatinine level alone. Using a combined endpoint of serum creatinine and clinical events, 22 patients (1.7%) experienced a grade 1 event, and one patient (< 0.1%) experienced a grade 4 event. Using a combined endpoint of glomerular filtration and clinical events, 128 patients (9.9%) experienced a grade 3 event, and 7 patients (< 0.1%) experienced a grade 4 event. A past

history of renal disease, abnormal baseline GFR, hypertension, and concurrent use of other nephrotoxic medications were identified as predictors of renal insufficiency. The serum creatinine elevations in CHORUS are comparable to those in other populations, and the proportion of patients with extreme grade 4+ glomerular filtration values is comparable to results seen for grade 4+ serum creatinine in other studies. However, 9.9% of patients on study met the criteria for grade 3 renal dysfunction using the GFR criterion, which corresponds with the National Kidney Foundation's definition of chronic kidney disease. Glomerular filtration is therefore probably a better indicator of renal function in HIV.

Despite tenofovir's passable renal safety profile as demonstrated in clinical trials, various case reports and observational data continue to suggest a risk of nephrotoxicity. Joel Gallant presented data from a large observational cohort at Johns Hopkins in which patients starting TDF ($n = 344$) or an alternate NRTI ($n = 314$) were compared for rates of decline in renal function. The study measured change from baseline creatinine clearance, determined by the Cockcroft-Gault equation using the average of 2 serum creatinine levels obtained within 90 days prior to initiation of therapy, over a period of one year. Median baseline creatinine and creatinine clearance were 0.8 mg/dL and 117.5 mL/min, respectively, with no differences between groups. Median creatinine change was +0.15 and +0.10 in the TDF and NRTI groups, respectively ($p = 0.01$); median creatinine clearance change was -13.35 and -7.5 mL/min ($p = 0.005$). In a multivariate analysis only TDF use and lower CD4 cell count were associated with decline in creatinine clearance. Hypertension, other concomitant antiretroviral therapy, viral load, previous use of adefovir, age, sex, race, and HIV transmission risk group had no such association. The TDF-associated changes were small but statistically significant; their clinical significance is unclear. The data in any case confirm the need to continue careful monitoring of renal function in patients taking TDF.

Bone Complication

Another potential complication associated with HAART is the loss of bone mineral density. Based on 144-week follow-up data from the Gilead 903 study, Powderly et al. compared rates of osteopenia and osteoporosis in previously naive participants started on either TDF ($n = 299$) or d4T ($n = 301$). Background therapy was composed of 3TC and efavirenz. All participants underwent dual x-ray absorptiometry (DXA) scans of the hip and spine at baseline and every 6 months for measurement of bone mineral density. Both groups experienced decreases in bone mineral density. The decreases were similar at the hip, but there were small differences in lumbar spine bone density change that favored the d4T arm. However, the incidence of osteopenia and osteoporosis was quite similar in the two groups.

Tenofovir's role in the development of osteopenia was confirmed by another study (Jacobsen et al.), which also implicated didanosine. This study evaluated the association between time on specific HAART and change in bone mineral density over time in 403 HIV-positive individuals (302 men, 101 women). DXA measurements were obtained yearly, with 903 intervals over a mean of 3.1 years. The median annualized change in total bone mineral density was -0.0187% per month ($p < 0.0001$), with a loss of 0.22%

over 1 year and a loss of 0.68% over 3 years. In addition to longer time on TDF or ddI, the number of years since HIV diagnosis and bilirubin values greater than 2.0 mg/dL were associated with bone density loss. Longer duration of d4T use, on the other hand, was found to have a slightly protective effect.

DEEKS (two)

HIV-1 Superinfection

Returning to the dais, Dr. Deeks said he had decided that, given the conference's traditional focus on pathogenesis, he should cover some of the related highlights. He noted that quite a bit of attention had been devoted to the issue of superinfection. Superinfection, it seems, is indeed a real phenomenon. Julie Overbaugh presented, in a plenary session, findings from a cohort of 20 women in Kenya with primary HIV infection, subtype C or D. Subtype A is more prevalent; thus, these subjects would be more likely to experience intersubtype superinfection. Three cases of subtype A superinfection were found in 70 person-years of follow-up, a period in which 6 cases were expected. This corresponds to the approximate rate of seroconversion from HIV-negative to positive in a similar population, indicating that prior infection probably does not provide a barrier to reinfection.

Bob Grant and colleagues presented information from the UCSF Options study, which follows individuals in the Bay Area with primary HIV infection. The study team followed 104 antiretroviral-naïve subjects, looking for the development of superinfection by performing serial genotypes. They found 4 cases of new infection, or 2.1 per 100 person-years, which is slightly less than would be expected among persons who were not HIV-infected. It has clinical significance, in that at the time of the shift from the first to the second virus – as seen in Dr. Grant's cases – the viral load increases. This makes sense, Dr. Deeks noted, since theoretically the incoming virus, to establish itself, has to be more fit than the strain already present. Also of clinical significance, Dr. Grant described one case of a patient with wild-type virus who, approximately 6 months after primary infection, developed NNRTI-resistant virus. Potentially, then, there are serious implications, and being HIV-positive does not seem to confer protection. We should keep in mind, however, that superinfection appears to occur in the context of recent infection (6-24 months); it has not been reported in chronically infected or antiretroviral-experienced patients.

Antiviral Host Responses

An emerging focus in the field of HIV-1 virology is the host antiviral effect potentially mediated by APOBEC3G. This topic dominated the conference's plenary sessions and, Dr. Deeks predicted, it will do so again next year. APOBEC3G is a protein, a cytidine deaminase that mutates nucleotides (G to A) in single-stranded viral DNA. The host protein APOBEC3G is packaged in virions as the virus is produced and, as the virions infect new cells, APOBEC3G is thought to cause mutations as RNA is made into DNA, making subsequent viruses defective. This is a very potent antiviral activity within the human cell, and it appears to be relevant to a wide variety of viruses. (This finding provides an example of advances in HIV research leading to secondary gains in other

fields: a recent article in *Nature* described the way APOBEC3G cleans up the endogenous retroviruses that we all have.) Unfortunately, HIV contains a protein, Vif, that binds to APOBEC3G and modifies it such that it is targeted for destruction by the cell itself. Efforts to describe this neutralization effect are ongoing.

Another development in the study of the host-virus dynamic is the identification of the host protein TRIM5 α , an enzyme of unclear function that somehow inhibits viral activity post-entry (likely by altering HIV uncoating before reverse transcriptase). We should expect to hear much more about it at CROI 2006.

How does HIV cause AIDS?

Dr. Deeks recommended, for anyone interested in the fascinating riddle of how HIV causes AIDS, the webcast of Daniel Douek's plenary talk, "Making Sense of HIV Disease Pathogenesis." His ideas are entertaining and provocative and, in Dr. Deeks' opinion, unlikely to prove valid; they do, however, advance the field a fair amount. Essentially, of course, we do not know how HIV causes AIDS, but we do know a number of things from presentations at this conference and other recent forums. Acute HIV infection (within a few weeks) is associated with rapid depletion of CCR5+ memory CD4 T cells in intestines, where a high proportion of cells are activated. Approximately 40% of CD4 T cells in the gut contain HIV within weeks after infection; within months, 50% of the total body population of CD4 T cells is depleted as a result of this massive destruction in the gut. This is clearly seen in macaques and 2 separate studies have indicated that it happens in humans as well. This depletion in the intestine continues at a much higher rate than in other locations, developing into chronic infection. With the introduction of HAART viral replication decreases but the CD4 cell depletion persists. There may be a profound total body lymphopenia even when CD4 cell counts in the blood have normalized. One might wonder what this says about David Ho's theory that HIV causes AIDS simply by killing T cells ("It's the virus, stupid."); given that the number of infected CD4 cells in the blood is extremely low, perhaps there is a more indirect mechanism. Moving forward in time, the amount of infection in the intestine seems insufficient to account for the continued loss of CD4 cells. Instead, during chronic infection HIV-associated immune activation appears to result in the accumulation of HIV-specific and other memory T cells within lymph nodes, causing inflammation and the progressive degradation of the lymphoid system. So the progression of disease appears to be the consequence of a number of factors. The discovery of the chaotic destruction during acute infection, though, has been somewhat a shock to those who have seen the data.

LAMPIRIS (two)

Hyperlipidemia and cardiac complications

"From the sublime to the mundane," said Dr. Lampiris, referring to the return from the provocative discussion of pathogenesis to his topic, metabolic complications. He began with hyperlipidemia, specifically the prospect of improving lipid levels by switching the current PI to atazanavir. In short, as borne out by two studies, switching to RTV-boosted ATV is associated with improvements in lipids and the improvements are greater with

ATV alone. Martinez et al. presented data from the Spanish ATV expanded access cohort study ($n = 255$), in which patients with baseline hyperlipidemia (triglycerides >500 mg/dL, total cholesterol >200 mg/dL, or LDL >130 mg/dL) were followed after switching from one PI-based regimen to another that contained ATV/r. After 6 months, 58% had viral loads <500 copies/mL, compared to 45% at baseline; median changes in triglycerides, total cholesterol and LDL were -18% , -10% and -10% , respectively. Approximately a third of the patients were able to discontinue lipid-lowering therapy. Fourteen patients experienced jaundice secondary to ATV/r, but only 3 required drug discontinuation. The second study, presented by Sension et al., was an evaluation of lipid profile changes 12 weeks after switching from any PI (boosted or not) to unboosted atazanavir. Two hundred forty-four virologically suppressed subjects with LDL >130 mg/dL were randomized to continue their current PI or PI/r or to switch to ATV. Forty percent were on a PI/r, 20% on Kaletra. The study excluded those with prior viral rebound on PI therapy. Comparing the “switch arm” to the “continuation arm” at 12 weeks, the study team found that the changes in blood levels on ATV were -15.2% for LDL, -17.4% for total cholesterol, and -34.8% for triglycerides – significantly better, as expected, than the changes seen with ATV/r. In the switch arm, 33% reached LDL levels <130 mg/dL, versus 14% on the continuation arm. There was 1-2% virologic failure in each arm; 48-week data is pending.

Of course, hyperlipidemia is but one cardiovascular risk factor. The D:A:D study, from which two abstracts were presented, involves observational follow-up of 23,441 patients in the US, Europe and Australia for cardiac complications. El-Sadr et al. assessed the incidence rate of first prospective MI, as well as relative rates of MI risk factors; the rates were compared with those of HIV-negative individuals matched for age and sex. The mean exposure time to combined ART in this group was 4.46 years, with 4,161 patients naive at entry. MI rates (per 1,000 patient-years) increased from 1.39 in those not exposed to ART, to 2.53 among those exposed to ART for less than 1 year, to 6.07 among those with ≥ 6 years of ART exposure. The relative risk for MI was similar for men and women with ART exposure, as well as in younger and older patients. A 1.17-fold increased risk of MI per additional year of HAART exposure was observed. Dyslipidemia explained part but not all of the association of combined ART with risk of myocardial infarction. Sabin et al. identified prior CVD, smoking, and family history of MI as major risk factors, but noted that, despite an increasing percentage of patients at high CVD risk over time (December 1999 to January 2004), the actual risk of MI has decreased. This decrease is possibly related to the use of different ART as well as treatment for hyperlipidemia.

Viral hepatitis coinfection

Dr. Lampiris concluded with two presentations on hepatitis coinfection. Peters et al. reported results of a study comparing TDF and adefovir for treatment of 52 HIV/HBV coinfecting patients. These results may not seem clinically relevant, Dr. Lampiris commented, since most local practitioners use TDF for such individuals; nonetheless, the study does answer the important question of whether TDF's anti-HBV effect is inferior to that of adefovir. Eligible patients had HBV DNA levels $>100,000$ copies/mL and HIV RNA levels $<10,000$ copies/mL. They were randomized to either adefovir 10 mg QD or

TDF 300 mg QD, and median follow-up was 75 weeks. The study was stopped prematurely because of low enrollment, but 48-week analysis showed mean HBV DNA reductions of 4.4 log₁₀ in the TDF arm and 3.2 log₁₀ in the adefovir arm. Though TDF was associated with superior response, the study was powered to assess non-inferiority, not superiority.

Sulkowski et al., from Johns Hopkins, reported on the risk of fibrosis progression in HIV/HCV coinfection. Within the current HCV treatment guidelines there is the option to defer HCV therapy in those patients with minimal fibrosis. To assess whether or not these guidelines should apply to HIV/HCV coinfection, the Hopkins study followed 67 coinfecting patients at one clinic with paired biopsies; mean time between biopsies was 2.83 years. The paired biopsies were evaluated at the same time by a single pathologist blinded to biopsy sequence, and scored according to the Ishak scale, F0 (no fibrosis) to F6 (cirrhosis). Surprisingly, 28% of patients experienced a ≥ 2 stage increase (the criterion for fibrosis progression), suggesting that treatment guidelines may need to be revised for coinfection and patients may require more aggressive follow-up. Increased scores were associated with HIV RNA levels >10,000 copies/mL and elevated AST levels at baseline. Dr. Lampiris noted that the study sample had disproportionate numbers of African Americans (86%) and active alcohol users (27%), so that results may not translate to the general population. Additionally, the sample did not obtain second biopsies on all patients with baseline fibrosis; the paired biopsies that were studied occurred in the context of clinical care, i.e., in the presence of transaminitis or some other indication of liver disease. The study findings may thus overstate the risk for progression of fibrosis.

FOLLANSBEE (three)

Capravirine

In his concluding remarks, Dr. Follansbee first spoke briefly of Pfizer's findings on capravirine (CPV) safety, tolerability and efficacy. CPV is a second generation NNRTI with *in vitro* activity against HIV-1 strains resistant to approved NNRTIs. Pesano et al. presented results of a 24 week study in which NNRTI-experienced, PI-naive patients were randomly assigned 1:1:1 to CPV 700 mg, CPV 1400 mg or placebo, in addition to nelfinavir and 2 optimal NRTIs as determined by genotype. The rates of discontinuation due to adverse events were 12%, 7% and 8%, respectively. Respective proportions of subjects with viral load <400 copies/mL at week 24 were 46%, 59% and 43%; proportions with viral load <50 copies/mL were 40%, 52% and 39%. Differences were not statistically significant but were, according to the abstract authors, clinically significant. Nevertheless, Dr. Follansbee dryly noted, we probably won't see many CPV studies in the near future.

MTCT

Dr. Follansbee finished with a discussion of 3 presentations on mother-to-child-transmission (MTCT) prevention, single-dose administration of nevirapine (sdNVP), and nevirapine resistance. As previously reported, the sdNVP strategy is effective in MTCT prevention; however, NVP-associated mutations are quickly evident in both mothers and

children following the intervention. Susan Eshleman and the HIVNET 012 protocol team presented data on the persistence of the K103N mutation in 9 women and 5 children in plasma collected before and at 12 and 24 months after dosing. Population sequencing at 6-8 weeks by the ViroSeq genotype assay detected the mutation in 8 of 9 women and 2 of 5 infants. A more sensitive point mutation assay, LigAmp, with a lower limit of detection of 0.08%, detected K103N at a level above 0.1% in 8 of 9 women (mean = 14%) and 4 of 5 infants (mean = 12%). At 12 and 24 months, the ViroSeq assay did not detect the mutation in any of the samples; however, the LigAmp assay found K103 at low levels in 3 women (0.8%, 1.3% and 3.5%) and one infant (1.5%), and a phenotypic assay confirmed K103N's presence in the samples.

A similar study was conducted by Palmer et al. in 17 South African women with HIV-1 subtype C who were participants in an sdNVP MTCT trial. The team compared detection of the K103N and Y181C mutations among longitudinal samples by standard genotyping, which does not capture variants that comprise <25% of the virus population, and by an RT-PCR assay allele-specific for K103N and Y181C. Subjects were divided into 2 groups by standard genotyping: group 1 ($n = 8$) included those with NNRTI-associated mutations at 6 weeks and 6 months, but not at 12 months, and group 2 ($n = 9$) had mutations at 6 weeks but not 6 months. Allele-specific RT-PCR testing detected 103N or 181C mutants in 7 of the eight 12-month samples in group 1, which had been negative by standard genotype; frequencies ranged from 0.25% to 16%. Similarly, the assay found 103N mutants in 7 of nine 6-month samples in group 2 (also negative by standard genotype), with frequencies from 0.9% to 10%.

Finally, Neil Martinson et al. reported preliminary results of a comparison of MTCT rates among women in 13 South African clinics given sdNVP in 2 successive pregnancies (cases) versus those receiving the single dose for the first time (controls). Case:control enrollment was 1:2, and controls were matched for age, clinic and enrollment time. Maternal viral load, CD4 cell count and HIV resistance level (determined by standard genotyping) were measured prior to sdNVP and at 6 weeks post-partum. An HIV-1 DNA-PCR assay was performed to determine MTCT at 6 weeks. Among the 68 cases and 118 controls that were evaluable, one mother (a case) showed baseline NVP resistance; she did not transmit HIV to her child. At 6 weeks, however, 38.2% of cases and 50.0% of controls showed resistance. HIV transmission rates were 13.7% and 4.2%, respectively - not statistically significant within these preliminary findings, but suggestive of a slightly higher risk associated with second sdNVP administration. The authors conclude that a second administration is safe; however, the study is ongoing and this conclusion may well be reversed.

These results are relevant to local practice, said Dr. Follansbee, since most clinicians base treatment decisions on results of population sequencing that may not detect circulating mutant strains. Given that it is still unclear how to discontinue NNRTI therapy without risking the development of resistance, it is important to remember that a standard genotype showing no mutations does not necessarily indicate that reusing an NNRTI is safe.

DEEKS (three)

Resistance and residual activity

Dr. Deeks concluded with coverage of various presentations that he considered “highlights.” First was the Jaguar study (Bates et al.), in which patients failing combination antiretroviral therapy were randomized to receive ddI ($n = 110$, 98 evaluable) or placebo ($n = 58$) to determine whether phenotypic susceptibility is a predictor of virologic response to ddI. The study found that ddI was quite effective, even when resistance was present at baseline. Virologists compared the phenotype at the point where the single drug was added with the virologic response, and were able to find a fold-change (FC) cut-off: an $FC \leq 1.3$ correlated with a good response to ddI, whereas an $FC \geq 2.2$ correlated with a terrible response. With response defined as a $>0.5 \log_{10}$ drop in HIV RNA, the probability of response was 83% for $FC \leq 1.3$ and 29% for $FC \geq 2.2$. Thus, with a phenotype a virologist can determine with some certainty the antiviral activity of ddI based on the IC_{50} . FC cut-offs have also been defined for abacavir and lopinavir; research with other agents is ongoing. When this work is complete, the phenotype promises to be much more precise and reliable.

The issue of “which drugs work and why” is an interesting one, and thus far, as with the Jaguar study, investigative approaches have involved interrupting the single agent in a failing regimen. A team at SFGH has, for instance, looked at patients resistant to T-20 ($n = 22$) and found that they experienced a $0.1 - 0.2 \log_{10}$ increase in viral load after interruption of the drug, a statistically but not clinically significant result. Decrease in CD4 cell count was consistent with that seen prior to the interruption. The indication is that, once T-20 resistance develops, the drug may not have residual antiviral activity. This is in sharp contrast, said Dr. Deeks, to results that he and others have seen with other therapeutic drug classes. T-20’s genetic barrier to resistance appears to be quite low, with drug failure occurring as early as week 2. The associated mutations do seem to have a dramatic effect on viral fitness, the clinical significance of which is not yet clear.

AZT, 3TC and tenofovir

Another controversial issue at the conference was that of triple nucleoside therapy. Current treatment guidelines quite clearly advise against such regimens; however, some practitioners continue to believe that the particular regimen of AZT, 3TC and TDF is effective, because K65R, the mutation for TDF, lends extraordinarily potency to AZT and cannot coexist on the same virus with TAMs. The three drugs together provide a strong genetic barrier to viral evolution. Is this an appropriate regimen, though, considering what we know about Trizivir and the higher risk of virologic failure associated with it? The question is addressed in DART, a large trial in Africa comparing intensive vs. clinical monitoring and continuous vs. intermittent therapy in treatment-naïve, symptomatic patients with CD4 cell counts $<200 \text{ cells/mm}^3$. Of 3263 patients, 76% were given AZT+3TC+TDF as a first-line regimen; 100 patients from each of three sites (2 in Uganda, 1 in Zimbabwe) were followed in a 24-week virologic substudy. Results from the Ugandan site ($n = 200$) were reported. The median CD4 cell count was $100/\text{mm}^3$, and median viral load was 336,000. At week 24, 51% of this very sick population had achieved HIV RNA levels $<50 \text{ copies/mL}$ and 68% had levels <400

copies/mL – quite impressive data. The debate on triple-nucleoside regimens continues, but many investigators, especially those in Africa, are arguing strongly for randomized trials of the AZT+3TC+TDF combination. Its potential benefits include limited pharmacokinetic interactions with TB medications, limited hepatic toxicity, the apparent absence of teratogenicity and, possibly, the preservation of treatment options.

Immune-based therapies

Next Dr. Deeks briefly discussed a presentation on an 8-week course of cyclosporin (CsA) as an immune-based therapy in acute HIV infection (Khonkarly et al.). Decreasing immune activation in the setting of primary HIV infection is beneficial both virologically and immunologically. This open-label study compared the effects of HAART with CsA, which down-regulates the genes that are essential for CD4 lymphocyte activation, to those of HAART alone. The two groups achieved similar reductions in viral load, although more in the HAART+CsA group ($n = 34$) reached <50 copies/mL than in the HAART-only group at weeks 18, 24 and 36. Mean CD4 counts in the CsA group were substantially higher than those in the HAART-only group at weeks 1, 2 and 4 (428 vs. 109, 379 vs. 217, and 373 vs. 201, respectively). Significantly, this rapidly induced change was maintained over time: mean CD4 counts at week 120 were 1115 vs. 888.

Levy et al. reported on long-term virologic control following therapeutic vaccination, with data from the second phase of the ANRS-093 study. In this trial, patients on HAART with CD4 counts >350 cells/mm³ and plasma viral loads <50 copies/mL were randomized to HAART alone ($n = 37$) or HAART combined with vaccine (ALVAC vCP1433 + HIV-Lipo-6T) administered at weeks 0, 4, 8 and 12 and followed by 3 cycles of subcutaneous IL-2 at weeks 16, 24 and 32 ($n = 33$). At week 40, HAART was interrupted and not resumed as long as viral load remained $<50,000$ copies/mL at 4 weeks and $<10,000$ copies/mL every 4 weeks subsequently. The second phase, weeks 52-100, followed patients who had resumed HAART and had viral loads <50 copies/mL. These patients again interrupted therapy, with the same indication for resumption of HAART as in the first phase. Median time off HAART was 177 days for the vaccine/IL-2 group vs. 89 days for the control group; the proportion off HAART at week 100 was 38% vs. 19%, respectively. If these data are correct, they may be the first hard evidence that one can actually improve the immune system's capacity to control the virus.

Integrase inhibitor

Finally, Dr. Deeks covered a report (Susan Little et al.) on the safety and efficacy of Merck's integrase inhibitor, L-000870810, in short-term monotherapy. Merck halted development of the drug several years prior to this study due to concerns about hepatotoxicity in dogs. They maintain that their new formulation has greater potency and is less toxic, a claim supported by the results presented. This was a 10-day study in which patients were randomized to L-000870810 at 200 mg ($n = 7$) or 400 mg ($n = 17$) or to placebo ($n = 6$). The sample included patients both naive and experienced (off therapy ≥ 3 months). The drug was quite effective at both doses, with 1.73 and 1.77 log₁₀ viral load reductions at day 10, respectively; it was also well-tolerated, with no serious adverse events and no AE-related drug discontinuations. Thus, a new therapeutic class of antivirals is on the horizon, which bodes well for the future.

LAMPIRIS (three)

Pharmacokinetics and pharmacogenomics

Dr. Lampiris began the final segment of his presentation with a discussion of the interaction between atazanavir and omeprazole. ATV now has a black box warning on its label advising against coadministration of the two drugs. Omeprazole (OMP), a proton-pump inhibitor, suppresses acid secretion and thereby increases gastric pH; unfortunately, ATV's solubility is pH-dependent and its absorption decreases as pH levels go up. Agarwala et al. gave atazanavir/ritonavir to 48 healthy subjects for 10 days, then randomized them to 3 groups: (1) ATV/r 300/100 mg + 40 mg OMP, (2) ATV/r 300/100 mg + 40 mg OMP + cola, and ATV/r 400/100 mg + 40 mg OMP. Follow-up continued for 10 days, with PK samples drawn at days 10 and 20. Results showed that ATV exposures (AUC, C_{max} and C_{min}) were substantially reduced across arms; giving cola and increasing ATV dose did not improve the PK interaction. Dr. Lampiris noted that the current recommendation for coadministration of ATV and H₂ blockers is to dose them 12 hours apart, although, to his knowledge, data on the PK interaction is not yet available.

Another study (Haas et al.) looked at genetic variants in the P450 2B6 isoenzyme and associated differences in the metabolism of nelfinavir and efavirenz, a quite relevant topic given current concerns about the variability of EFV's absorption rates by race. The study team examined repository specimens from 504 participants in ACTG 384 who took EFV and/or NFV; these included 49% Caucasian, 31% African American and 19% Hispanic subjects. The single nucleotide polymorphism CYP2B6 G516T was associated with higher plasma EFV AUC_{24h} values in all subjects and in white, black, and Hispanic subpopulations. However, although genetic polymorphisms were associated with increased EFV exposure, higher drug levels were not associated with differences in virologic response at week 48. Related findings were presented by R-Novoa et al., who found that CYP2B6 polymorphisms correlated with increased risk of efavirenz CNS toxicity. They studied 111 HIV-positive Caucasians who started EFV in 2003: 49% were wild-type (G/G), 44% were heterozygous (G/T), and 7% were homozygous (T/T). Mid-dose plasma measurements were taken at week 12, and toxic levels of efavirenz were detected in 40% and 19% of patients with T/T and G/T alleles (versus none among G/G patients). Evidence of CNS toxicity was found in 30% and 32%, respectively (versus 4% among G/G patients). So these T-containing polymorphisms are not predictive of virologic response, but they are associated with greater exposure to EFV and higher risk of related CNS toxicity. Notably, however, the latter study found that one-fifth of G/G patients are exposed to sub-therapeutic EFV levels, which may represent a higher risk of virologic failure on longer follow-up.

Finally, Ritchie et al. performed a nested case-control study among a cohort of 445 patients on their first NNRTI-containing regimen. Of this group, 30 developed hepatotoxicity (ALT or AST >5x upper limit of normal or bilirubin >3.5 mg/dL), and 20 of this sub-group (11 nevirapine and 9 efavirenz cases) had DNA available for testing. There were 50 controls matched according to NNRTI, age, race, and hepatitis C status.

The sample consisted of 23% women, 77% men, 19% African Americans, and 81% Caucasians. The four single nucleotide polymorphisms analyzed were CYP2B6 G516T and C1459T, CYP3A4 A392G, and MDR1 C3435T. The findings were (1) that the T allele at MDR1 C/T correlated with decreased risk of NVP toxicity and (2) that the interaction of either CYP2B6 variant with the MDR1 variant was predictive of hepatotoxicity with 70% accuracy.

These pharmacogenomic data represent a significant advance. Perhaps within 5 years, said Dr. Lampiris, we will have the ability to predict hepatic toxicity with baseline genotyping and tailor individual therapy accordingly.

CONCLUSION

Dr. Abrams, on behalf of the Community Consortium, thanked Drs. Follansbee, Deeks and Lampiris as well as the evening's sponsors. He also took a moment to note Dr. Wally Krampf's imminent departure from San Francisco and to recognize his many years of service to the Consortium and the HIV community. He observed, as well, that Dr. Krampf's exodus creates an open slot on the Consortium's Executive Advisory Board, and he invited interested individuals to speak with him about serving in that role.

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