

RAPIDREPORT

XIII international
HIV DRUG RESISTANCE
workshop *June 8-12, 2004 • Tenerife, Canary Islands*
basic principles & clinical implications

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DESCRIPTION

This Rapid Report is a summary of the lectures and discussions presented at the XIII International HIV Drug Resistance Workshop. At the workshop, leading laboratory and clinical scientists presented their latest research, which often results in “paradigm” shifts in antiretroviral therapy. The Workshop is renowned for the quality of the data presented and the depth of the scientific interaction and debate.

OBJECTIVES

- ♦ Provide an update on resistance to new antiretroviral agents
- ♦ Review mechanisms of HIV drug resistance
- ♦ Present topics specific to HIV pathogenesis, fitness and resistance
- ♦ Expand the scope of the clinical implications of resistance
- ♦ Present new developments in epidemiology
- ♦ Introduce new resistance technologies and interpretations

STATEMENT OF NEED

The content of the XIII International HIV Drug Resistance Workshop was determined by assessment of educational need, including feedback from prior programs and new medical knowledge.

TARGET AUDIENCE

This summary was designed to interest physicians, clinicians, scientists and clinical researchers in the HIV resistance arena.

ACCREDITATION

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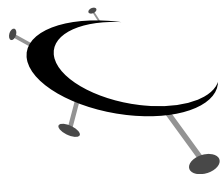
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Lee Bacheler	Employee: Virco Presentation will mention VirtualPhenotype™ analysis of HIV resistance
Marie-Laure Chaix	No Significant Relationship Disclosed
Steven Deeks	Research Support: ViroLogic, Hoffman La Roche, Trimeris, Pfizer Ad hoc advisor: GlaxoSmithKline, Bristol-Myers Squibb
Susan Eshleman	Research Support: Applied Biosystems, ViroLogic, Celera Diagnostics, Abbott Laboratories, Boehringer Ingelheim Consultant: GlaxoSmithKline, Applied Biosystems, ViroLogic, Abbott Laboratories Presentation will discuss use of single dose nevirapine for prevention of HIV mother-to-child transmission
Huldrych Günthard	No Significant Relationship Disclosed
Jennifer Hammond	Employee: Pfizer
Richard Harrigan	Ad Board or Grant Applications: Virco, ViroLogic, Roche
Daria Hazuda	Employee: Merck Service Provider: ViroLogic
Wei-Shau Hu	No Significant Relationship Disclosed
Jeffrey Johnson	No Significant Relationship Disclosed
Nathaniel Landau	Consultant: ViroLogic, Trimeris
Jeffrey Lifson	Presentation will describe use of Tenofovir (PMPA; Gilead Sciences) in studies in non-human primate models
Pin-fang Lin	Employee: Bristol-Myers Squibb
Susan Little	No Significant Relationship Disclosed
Frank Maldarelli	No Significant Relationship Disclosed
David Margolis	Research Support: Gilead Sciences, Bristol-Myers Squibb, Roche, Trimeris, Tibotec, Boehringer Ingelheim Stockholder: Gilead Sciences, GlaxoSmithKline, Bristol-Myers Squibb, Merck, Abbott Advisory Board: Gilead Sciences, GlaxoSmithKline, Bristol-Myers Squibb, Trimeris
Douglas Mayers	Employee: Boehringer Ingelheim Presentation will include Tipranavir
Peter Meyer	No Significant Relationship Disclosed
Michael Miller	Employee: Merck & Co., Inc. Presentation will include data from ViroLogic
Monique Nijhuis	Research Support: Roche
Michael Parniak	No Significant Relationship Disclosed
Vinay Pathak	No Significant Relationship Disclosed
Christos Petropoulos	Employee, Officer, Shareholder in ViroLogic Several meetings supporters are clients of ViroLogic
Deenan Pillay	Consultant: GlaxoSmithKline, Gilead Sciences, Bristol-Myers Squibb, Roche
Assia Samri	No Significant Relationship Disclosed
Mike Westby	Employee: Pfizer Presentation will include the antiretroviral drug in development: CCR5 Antagonist, "UK427,857"

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Five years ago Peter Meyer and Michael Parniak showed up in the sun-baked climes of southern California to tell Resistance Workshop attendees they had—in separate studies—divined the clockworks that make resistance to AZT tick. Nice, clinicians may concede, but how does that help me? This year Meyer and Parniak showed up in the sun-baked climes of the Canary Islands with an answer: They had—in separate studies—divined the first steps in unwinding this resistance mainspring (see section 2 below).

And their reports were not the only milestones on the switchback-swagged road to progress against HIV. Since its inception soon after the discovery of resistance to AZT, the Resistance Workshop has become the premier forum for researchers in this field. Once a year they vie to unveil unpublished data and subject it to the scrutiny of rivalrous colleagues and collegial rivals. Whereas the first Resistance Workshop featured a few score reports, the meeting's

latest edition attracted 256 submissions, from which the Scientific Committee plucked 9% for oral sessions and 56% for posters. But poster presenters dare not relax, since the organizers also elect reviewers to summarize pertinent poster findings and challenge researchers to defend their conclusions.

The Workshop sorts reports into six broad areas:

1. Resistance to new antiretroviral agents
2. Mechanisms of HIV drug resistance
3. HIV pathogenesis, fitness, and resistance
4. New resistance technologies and interpretations
5. Epidemiology
6. Clinical implications of resistance

At this year's sessions, investigators mapped out resistance patterns to a host of new agents, including attachment, entry, and integrase inhibitors, and more familiar reverse transcriptase and protease inhibitors. Attendees learned how a new route to protease inhibitor (PI) resistance may explain some PI failures without protease mutations, how RNase H mutations may confer high-level resistance to AZT and d4T, and how HIV may manage to escape entry inhibitors. In the pathogenesis session, researchers proposed tactics for rousing latent HIV from resting cells without riling the cells themselves and gauged how readily HIV genomes may recombine.

Epidemiologists tracked rates of resistant virus in treated and untreated people (resistance to nonnucleosides is on the rise everywhere), while the tech experts mapped the impact of drug history on resistance, explained a novel assay that can read resistance in a dried blood spot, explored simultaneous resistance monitoring for HIV and hepatitis C virus, and rated top virologists on their interpretive skills. Clinically oriented attendees scrutinized results of a pilot trial in which one attractive efavirenz regimen fell flat and debated how much resistance affects mortality.

1. RESISTANCE TO NEW ANTIRETROVIRAL AGENTS

Scaling up to a higher resistance barrier? The search for antiretrovirals that tamp down replication of resistant virus continues—at a measured pace. Two Pfizer candidates, one

a PI and one a nonnucleoside (NNRTI), attracted some attention at the Workshop.

A PI labeled AG-001859 boasts a median 50% inhibitory concentration (IC_{50}) of 30 nM against certain PI-resistant viruses and remains 30% unbound by plasma protein. Although 1859 does not inhibit CYP3A4 (the pan-PI metabolizing enzyme), it is a CYP3A4 substrate.

Pfizer's Jennifer Hammond tested 1859 and current PIs against a panel of ViroLogic isolates, of which 52%, 36%, and 5% bore 1, 2, or 3 key PI mutations including D30N, G48V, I50V, V82A/T, I84V, and L90M; 82% of these isolates had more than 10-fold resistance to one or more marketed PIs. When pitted against resistant isolates comprising 80% of this panel, 1859 had a median IC_{50} of 75 nM (1.5-fold change in susceptibility relative to wild-type virus) versus 92 nM for saquinavir (17-fold change), 119 nM for lopinavir (15-fold change), 130 nM for amprenavir (3.4-fold-change), 278 nM for nelfinavir (17-fold change), and 323 nM for indinavir (9.3-fold change). The Pfizer/Agouron PI had good activity against virus resistant to five familiar agents (Table 1).

Table 1. Activity of AG-001859 against isolates resistant to other PIs		
	<i>Median (range) IC_{50} (nM)</i>	<i>Median (range) fold-change</i>
Amprenavir	100 (33 to 210)	4.8 (1.5 to 9.6)
Indinavir	51 (18 to 420)	3.1 (0.8 to 19.5)
Lopinavir	41 (9.3 to 170)	1.8 (0.4 to 7.7)
Nelfinavir	49 (13 to 420)	2.6 (0.6 to 19.5)
Saquinavir	62 (27 to 420)	3.2 (1.2 to 19.5)

After 200 days of serial passage, 1859 selected virus with 10-fold resistance relative to wild type and three mutations, which Hammond did not name. This drug is in phase 1 trials.

Efavirenz and nevirapine are potent and relatively tolerable nonnucleosides, but their low resistance threshold poses risks for people with faulty adherence. Analysis of two phase 2 studies suggests that resistance does not evolve as readily to the investigational NNRTI capravirine.

Researchers halted two phase 2 studies of capravirine plus nelfinavir and two nucleosides (NRTIs) in people with NNRTI experience when high doses caused vasculitis in animal studies (a side effect not seen when studies resumed in humans). Phase 2 trial participants who had pushed their viral load below 400 copies/mL could continue the regimen, and 36 of them did. Sixteen people (44%) maintained a viral load below 400 copies/mL during 41 to 51 months of therapy. Five people (14%) stopped the regimen because of side effects, 9 (25%) for reasons unrelated to study drugs, and 6 (17%) because of virologic failure.

Jennifer Hammond reported a median time to failure in these 6 people of 19 months (range 4 to 27 months). In 2 of the 6 no new mutations conferring resistance to capravirine or nelfinavir emerged during 11 and 15 months of therapy, and HIV did not become less susceptible to those drugs. Hammond did not have a genotype for 1 person with a virologic failure. Phenotypic results for that person showed decreased susceptibility to nelfinavir but not to capravirine or the NRTIs. Virus in the remaining three people lost susceptibility to capravirine and nelfinavir. These people had NNRTI resistance mutations at varied positions in reverse transcriptase: 101, 108, 190 and/or 188.

Hammond concluded that people with NNRTI experience can control viral replication for prolonged periods with a capravirine-containing regimen. Results of this study and earlier research suggest that a single mutation conferring high-level resistance to capravirine does not emerge in people taking this nonnucleoside—a trait that would distinguish it from efavirenz and nevirapine if it holds true in larger studies. The varied patterns of slowly emerging reverse transcriptase mutations that arise when capravirine fails suggest a higher barrier to resistance than with efavirenz or nevirapine. How long HIV will take to scale this barrier awaits completion of ongoing phase 3 trials.

—Hammond J, Jackson L, Graham J, Knowles S, Digits J, Tatlock J, Jewell J, Canan-Koch S, Patick AK. Antiviral activity and resistance profile of AG-001859, a novel HIV-1 protease inhibitor with potent activity against protease inhibitor-resistant strains of HIV. *Antiviral Therapy* 2004;9:S17. Abstract 13.

—Hammond J, Pesano R, Hawley P, Patick AK. Analysis of time of failure: genotype and phenotype from NNRTI-experienced patients treated with capravirine. *Antiviral Therapy* 2004;9:S19. Abstract 15.

How HIV may shrug off attachment of attachment inhibitors. Pin-Fang Lin (Bristol-Myers Squibb) detailed a clutch of mutations that confer resistance to small-molecule attachment inhibitors such as BMS-378806 and BMS-488043, which block binding of viral gp120 to cellular CD4. Amino acid substitutions selected by these attachment inhibitors map exclusively to env proteins. Cumulative results of these experiments indicate that resistance to this new class may involve multiple interactions between diverse regions of the gp120 and gp41 envelope proteins in a manner paralleling viral defenses against neutralizing antibodies.

Assessing resistance of viral variants selected by several attachment inhibitor analogs, Lin and colleagues found resistance-conferring mutations that span the entire viral envelope including more than 9 sites critical to CD4 binding. These mutation sets include:

- Double contact site substitutions lying in distant reaches of the CD4 binding pocket
- Substitutions overlapping epitopes of CD4 binding site antibodies and sites affecting viral susceptibility to soluble CD4
- Frequently occurring envelope changes at the V1V2 stem or lining the CD4 binding pocket
- Envelope substitutions at M434 and F423 (overlapping CD4i epitopes outside the CD4 binding pocket)
- Substitutions at the CCR5 binding sites in C4 and V3
- The V68A change near the N terminus of gp120
- Several changes at gp41

Only a W427V substitution appears to confer cross-resistance to the two BMS candidates. As would be expected, the agents have no cross-resistance to marketed reverse transcriptase or protease inhibitors. Cross-resistance studies involving other entry inhibitors are under way.

Passage studies in cell culture showed that resistance to these attachment inhibitors emerges with the alacrity of resistance to 3TC and nevirapine. But Lin noted that early results from human studies—which she did not

review—suggest this low barrier to resistance will not derail development of these agents. She also found that inhibition of soluble CD4 binding to gp120 by BMS-378806 is concentration dependent.

—Fan L, Zhou NN, Gong YF, Ho HT, McAuliff B, Fang H, Eggers B, Fang J, Li CB, Wang HG, Langley D, Kadow J, Lin PF. *In vitro* resistance profile of small molecule HIV attachment inhibitors. *Antiviral Therapy* 2004;9:S9. Abstract 5.

Send in the clones—as entry inhibitors? The antiretroviral enfuvirtide (T-20) blocks HIV fusion to CD4 cells by binding to a highly conserved region on HIV envelope gp41 labeled heptad repeat 1 (HR1). Michael Miller and Merck coworkers identified a human monoclonal antibody that appears to block HIV by the same mechanism. Their findings could lead to therapeutic monoclonal antibodies and may further vaccine development by pointing to immunogens that elicit neutralizing antibodies.

Miller selected bacteriophage-bearing human single-chain antibodies for their ability to bind to the gp41 HR1 region. One such antibody, D5, inhibited entry in a high-throughput HIV entry assay and blocked HIV replication in single- and multiple-cycle assays. D5 retained its antiviral potency after conversion to a human IgG1. Because D5 prevented binding of an epitope-tagged C peptide to HR1 *in vitro*, Miller proposed that D5 blocks HIV entry by the same mechanism as T-20.

In further experiments the potency of D5 IgG1 varied considerably in neutralizing a variety of HIV isolates, and some isolates proved almost completely resistant to the monoclonal antibody. Miller identified amino acid polymorphisms in the HR1 region of resistant isolates. But when he transferred these polymorphisms to a sensitive isolate, that isolate did not confer resistance to D5 IgG1. Biochemical studies showed that D5 IgG1 bound equally well to wild-type and polymorphic gp41 mimetics. These findings led Miller to conclude that resistance to D5 must be caused by regions of the envelope glycoprotein outside HR1.

Miller proposed that these findings offer proof-of-concept that human IgG can bind to gp41 HR1, that such an IgG can block HIV entry, and that one can design synthetic antigens bearing an HR1-derived neutralizing epitope. He believes this work shows that HIV's fusion machinery is

accessible to human IgG molecules, including those that may be elicited by a vaccine.

—Miller MD, Geleziunas R, for the HIV Antibody Discovery Team. *A human monoclonal antibody blocks HIV entry by a T20-like mechanism. Antiviral Therapy 2004;9:S13. Abstract 9.*

Where are the integrase inhibitors? Hopes for development of HIV integrase inhibitors turned to dismay when early research hit some high hurdles on the road to constructing worthy candidates. Three Workshop presentations showed that the road to development remains rocky.

Two types of long-hoped-for but difficult-to-develop integrase inhibitors, the diketo acids and the naphthyridines, have largely (but not completely) distinct resistance profiles. As with other antiretroviral classes, reported Merck's Daria Hazuda, at least one mutation may mediate cross-resistance to these two types of strand-transfer integrase inhibitors.

Earlier work by Hazuda and Merck's HIV-1 Integrase Discovery Team mapped diketo acid resistance to integrase residues 66, 151, 153, 154 and 155. When the Merck team turned their attention to a naphthyridine labeled L-870810, they uncovered an entirely new set of mutations at positions 74, 121, and 125. Recombinant viruses engineered to carry these mutations displayed a significant loss of susceptibility to naphthyridine integrase inhibitors, but not to the diketo acids. The converse proved true for all of the diketo acid-associated mutations except for the N155S change, which conferred 20-fold resistance to a Merck diketo candidate and 12-fold resistance to a Merck naphthyridine.

All mutations to both types of integrase inhibitors lay within the integrase active site. But the naphthyridine mutations traced out a region distinct from the one defined by the diketo acid mutations. This difference plus molecular modeling studies, Hazuda contended, suggest a molecular basis for the discordant resistance profiles and imply a role for N155 in maintaining the architecture of the integrase active site. As with other antiretroviral classes, she proposed, integrase inhibitors with differing resistance profiles should be developed.

One type of integrase inhibitor that may bear a resistance profile distinct from the Merck candidates could emerge from the styrylquinoline class described by Arnaud Chéret (BioalliancePharma). Unlike mutations conferring resistance to Merck's diketos and naphthyridines, those

that render HIV resistant to styrylquinolines sit outside the integrase binding pocket. A C280Y change yielded 5-fold resistance to FZ41, a styrylquinoline lead compound, and the V165I/V249I changes made HIV nearly 9-fold resistant to that compound compared with wild-type virus.

Describing another strand transfer inhibitor, Wataru Sugiura (AIDS Research Center, NIID, Japan) painted another picture of painstaking, if frustrating, effort. He tested 12,000 small-molecule compounds and found one—a carbazole derivative—entirely distinct from Merck's candidates. Using this compound as a basis to synthesize 15 constructs with different side chains branching from the carbazole backbone, he isolated 8 with potent activity against strand transfer. The IC₅₀s of these compounds ranged from 0.78 to 5.3 μM, but the 50% cytotoxicity concentration ranged from 1.9 to 5.04 μM. As a result, carbazole derivatives seem unlikely candidates for the clinic, but Sugiura proposed that they may be lead compounds for other agents.

Why has the gestation of a viable integrase inhibitor proved so painful? All antiretroviral development is painful, Hazuda said. Development of integrase inhibitors has been particularly paroxysmal, she suggested, because integrase is a novel enzyme that bears many dissimilarities from enzymes inhibited so far. As Chéret observed, unlike reverse transcriptase and protease, integrase has no host cell counterpart.

Another factor, Hazuda reminded colleagues, is that the antiretroviral approval bar stands several pegs higher today than it did in 1986 or 1996. Regulators, clinicians, and people with HIV now expect safer drugs that are easier to take.

—Hazuda D and the Merck HIV-1 Integrase Discovery Team. *A molecular model of HIV-1 integrase inhibitor resistance. Antiviral Therapy 2004;9:S5. Abstract 1.*

—Bonnenfant S, Zouhiri F, Chéret A, Leh H. *In vitro development of resistance against styrylquinolines of HIV-1 by emergence of integrase mutations. Antiviral Therapy 2004;9:S7. Abstract 3.*

—Yan H, Chiba T, Kitamura Y, Nishizawa M, Fujino M, Yamamoto N, Sugiura W. *Novel small-molecule compounds which inhibit strand transfer activity of HIV-1 integrase. Antiviral Therapy 2004;9:S5. Abstract 2.*

Slow--or no--resistance to a CCR5 antagonist. Serial passage studies of UK-427,857, Pfizer's investigational CCR5 antagonist, found that resistance to the drug emerges

slowly or not at all in cell cultures infected with primary HIV isolates.

High-level resistance to UK-427,857 evolved in three of six R5 primary isolate virus cultures that Pfizer's Mike Westby exposed to increasing concentrations of the drug. Despite emergence of resistance, two of the isolates (CC1/85^{res} and RU570^{res}) did not switch to the CXCR4 coreceptor, whereas the third resistant isolate (SF162^{res}) did learn to take the alternate pathway. Increased sensitivity to a CCR5-specific monoclonal antibody by CC1/85^{res} and RU570^{res} suggested to Westby altered envelope recognition of the external face of the CCR5 coreceptor. Infectivity of RU570^{res} (but not CC1/85^{res}) declined compared with the parent virus after resistance developed. Serial passage with three R5 isolates—92BR017, 92BR018 and 92BR023—could not generate resistance to UK-427,875.

Westby concluded that viral escape is difficult but possible with serial passage of primary isolates. Evolution to use R4 receptors can occur either in the presence or absence of UK-427,875. The slow emergence of resistance—or lack of resistance—in these studies, Westby proposed, indicates that the viruses studied have a selective advantage in sticking with the CCR5 receptor.

—Westby M, Smith-Burchnell C, Mori J, Lewis M, Mansfield R, Whitcomb J, Petropoulos CJ, Perros M. *In vitro* escape of R5 primary isolates from the CCR5 antagonist, UK-427,857, is difficult and involves continued use of the CCR5 receptor. *Antiviral Therapy* 2004;9:S10. Abstract 6.

2. MECHANISMS OF HIV DRUG RESISTANCE

Resistance to PIs without protease mutations. *In vitro* selection experiments with the protease inhibitor Ro-033-4649 demonstrated 6- to 8-fold decreased susceptibility to the drug without the emergence of resistance mutations in the viral protease. Instead, Noortje van Maarseveen (University Medical Center, Utrecht) detected nucleotide changes in the gag gene that may explain resistance to this PI, and perhaps to others.

The Utrecht group cultured HIV-1 HXB2 in SupT1 cells in the presence of increasing doses of Ro-033-4649. After 6 passages over around 120 day, the experimental PI selected virus with 6- to 8-fold resistance compared with wild-type virus, but the viral population contained no protease mutations. Sequence analysis of gag showed nucleotide changes at positions 436 and 437. Repeat experiments turned up substitutions in the same region. A site-directed

mutant containing changes at positions 436 and 437 proved 2- to 3-fold resistant to Ro-033-4649 compared with wild-type virus and 2- to 5-fold resistant to current PIs. But these constructs remained susceptible to AZT and nevirapine.

These nucleotide changes lie in the ribosomal frameshift site, which generates *pol* gene products. The same region carries the genetic code for the transframe protein (TFP) and the p7/p1, p7/TFP, and TFP/p6 protease cleavage sites. These viral changes, van Maarseveen theorized, may explain the observed drop in susceptibility to Ro-033-4649.

Earlier research described mutations at gag cleavage sites, but those mutations appeared to be compensatory changes, not primary mutations that directly confer resistance to PIs. Charles Boucher, senior investigator in the Ro-033-4649 study, suggested that the gag mutations this research identified may explain some PI failures in people with good adherence but without protease mutations.

—Nijhuis M, van Maarseveen NM, Schipper P, Goedegebuure IW, Heilek-Snyder G, Cammack N, Boucher CAB. Novel HIV drug resistance mechanism leading to protease inhibitor (PI) resistance in response to a high genetic barrier PI *in vitro*. *Antiviral Therapy* 2004;9:S42. Abstract 36.

Thwarting the workings of AZT resistance. Research over the past several years defined the mechanism of resistance to AZT as excision of the incorporated analog from the viral DNA chain, which allows continued viral DNA synthesis. That finding may have more than academic interest, if preliminary results of by two researchers who helped define this mechanism pan out. They asked the same question—can we target drug-resistant mutants to prevent their emergence or to suppress them if they do emerge?—and took different approaches to answering it.

Michael Parniak (University of Pittsburgh) identified a bisphosphonate labeled BPH-218 as an inhibitor of the AZT excision mechanism. BPH-218 inhibited adenosine triphosphate- (ATP-) and phosphorolysis-dependent excision of AZT monophosphate at a 50% inhibitory concentration (IC₅₀) of about 2 μM. But because this agent had little effect on reverse transcriptase-driven DNA synthesis, Parniak concluded that BPH-218 selectively inhibits the excision mechanism.

BPH-218 had only weak activity against thymidine analog mutant (TAM) virus resistant to AZT. When combined with AZT against TAM mutants, however, the bisphosphonate significantly enhanced AZT's antiviral

activity. AZT alone shows only marginal activity against TAMs, at a 50% effective concentration of about 3.5 μM compared with 0.3 μM against wild-type virus. But when AZT teamed up with BHP-218 its antiviral activity against TAMs improved to about 0.01 μM . That finding suggests that BPH-218 restores AZT activity against these resistant mutants.

The intriguing finding that BPH-218 also bolsters AZT activity against wild-type virus (by 28-fold) implies that AZT excision plays a role in replication of wild-type virus exposed to AZT. That could explain why thymidine analogs do not have as much antiviral activity against wild-type virus as one might expect. Parniak believes that the antiviral potency of bisphosphonates can be improved by standard medicinal chemistry approaches.

Peter Meyer (University of Miami) theorized that since nucleotide excision is a chemical reaction, it should be reversible. He tested the ability of dinucleoside tetraphosphates containing dideoxynucleosides (ddNp₄ddN) to inhibit DNA synthesis by wild-type or AZT-resistant HIV-1 reverse transcriptase.

When Meyer exposed virus to either ddTTP or ddTp₄ddT, DNA polymerization led to concentration-dependent declines in incorporated dNTPs because incorporation of the inhibitor into the DNA terminated the growing DNA chain. Wild-type reverse transcriptase proved more than 70-fold less sensitive to inhibition by ddTp₄ddT ($\text{IC}_{50} \sim 10 \mu\text{M}$) than by ddTTP ($\text{IC}_{50} \sim 140 \text{ nM}$). But reverse transcriptase bearing the AZT mutations 67N, 70R, 215Y, and 219Q was only 5-fold less sensitive to inhibition by ddTp₄ddT ($\text{IC}_{50} \sim 0.9 \mu\text{M}$) than to ddTTP ($\text{IC}_{50} \sim 0.2 \mu\text{M}$). And reverse transcriptase with the mutations M41L, 69S-AG, 210W, 211K, 214F, and 215Y proved equally sensitive to inhibition by ddTp₄ddT ($\text{IC}_{50} \sim 0.35 \mu\text{M}$) and ddTTP ($\text{IC}_{50} \sim 0.32 \mu\text{M}$).

These findings led Meyer to conclude that dinucleoside polyphosphates act as substrates for DNA polymerization by HIV-1 reverse transcriptase and incorporate themselves into reverse transcriptase containing AZT resistance mutations much more readily than into wild-type reverse transcriptase. Therefore, he proposed, dinucleoside polyphosphates have the potential to prevent or reverse development of AZT resistance mutations. His future work will focus on modifying the base, sugar, and phosphate groups of dinucleoside polyphosphates to increase inhibition of HIV-

1 reverse transcriptase and to optimize cellular uptake and stability of these compounds.

—Parniak MA, McBurney S, Oldfield E, Mellors JW. Bisphosphonate inhibitors of nucleoside reverse transcriptase inhibitor excision. *Antiviral Therapy* 2004;9:S32. Abstract 26.

—Meyer P, Smith A, Matsuura S, Scott W. Dinucleoside polyphosphates are novel inhibitors of HIV-1 reverse transcriptase with increased potency against enzymes containing AZT resistance mutations. *Antiviral Therapy* 2004;9:S37. Abstract 31.

Yet another mechanism for resistance to NRTIs?

Overcoming the excision mechanism of resistance to AZT, as suggested by the just-described work of Michael Parniak and Peter Meyer, may make AZT a more effective nucleoside. But unhinging that mechanism may not eliminate resistance to AZT and other reverse transcriptase inhibitors if a second resistance mechanism proposed at the Workshop proves true.

Vinay Pathak (HIV Drug Resistance Program, National Cancer Institute, Frederick) and colleagues at the University of Pittsburgh advanced a novel mechanism to explain nucleoside-mediated abrogation of HIV-1 replication that implies another route to resistance. They hypothesize that nucleoside therapy leads to an equilibrium between:

- Nucleoside incorporation into host DNA
- Excision of the nucleoside
- Resumption of DNA synthesis
- Ribonuclease (RNase) H activity

Degradation of the RNA template by RNase H before DNA synthesis resumes would uncouple the template and primer strands, halt reverse transcription, and so stop viral replication. This model predicts that mutations reducing the rate of RNA degradation will heighten resistance to nucleosides by prolonging the time for excision of incorporated nucleosides from terminated primers.

To test that prediction, Pathak used a single-cycle infection assay and the firefly luciferase reporter gene to rate the sensitivity of three viral constructs to AZT, d4T, ddI, 3TC, and efavirenz—virus with wild-type reverse transcriptase, virus with thymidine analog mutations (TAMs) in reverse transcriptase, and virus with two RNase H mutations (H539N and D549N).

D549N magnified resistance to AZT 10-fold and to d4T 2.6-fold, increases similar to those seen with TAMs. H539N swelled resistance to AZT 180-fold and to d4T 10-fold, about 9-fold and 4-fold higher than TAM-conferred resistance. These findings suggested that decreased RNase H activity can boost resistance to thymidine analogs independently of TAMs. The two RNase H mutations caused a modest spurt in resistance to ddI (about 1.9-fold) but did not affect viral sensitivity to 3TC or efavirenz. A viral clone from a patient with a nucleoside-resistant isolate had both the D549N mutation and TAMs, a finding suggesting that the RNase H mutation contributed to resistance.

Pathak and coworkers concluded that these results support their proposed mechanism for nucleoside-mediated abrogation of viral replication and suggest that RNase H mutations can be selected during antiretroviral therapy and confer high-level resistance to AZT and d4T.

—Nikolenko GN, Palmer S, Maldarelli F, Mellors JW, Coffin JM, Pathak VK. Mutations in HIV-1 RNase H domain confer high-level resistance to nucleoside reverse transcriptase inhibitors and provide novel insights into the mechanism of nucleotide excision-mediated drug resistance. *Antiviral Therapy* 2004;9:S26. Abstract 20.

How will HIV escape entry inhibitors? Agents that block viral fusion and entry offer the hope of locking HIV out of the cellular workshop where it copies itself into clusters of new virions. But no one doubts that the retrovirus will evolve strategies to sidestep these inhibitors, as it has done with reverse transcriptase and protease inhibitors. Work by ViroLogic's Christos Petropoulos suggested how HIV may plot its escape from diverse members of this new drug class.

Petropoulos gauged viral susceptibility to entry inhibitors with an envelope pseudovirus infectivity assay. He plotted susceptibility as percent inhibition versus \log_{10} drug concentration and defined susceptibility based on the 50% inhibitory concentration (IC_{50}) and percent inhibition at the highest drug concentration.

In the model formulated by the ViroLogic team, changes in IC_{50} best described susceptibility to fusion inhibitors such as enfuvirtide (T-20). Just as with protease and reverse transcriptase inhibitors, plots of increasing IC_{50} for virus resistant to fusion inhibitors compared with susceptible virus described a log-sigmoid inhibition curve. The ability

of fusion inhibitors to inhibit 100% of viral replication at high concentrations indicates a competitive mechanism of viral inhibition and escape.

On the other hand, resistance to certain receptor and coreceptor antagonists was often associated with a plateau in maximum inhibition reflecting an uninhibited fraction of virus. This inability to block 100% of replication at high coreceptor antagonist concentrations indicates a noncompetitive mechanism of inhibition and escape. In these situations, virus resistant to certain receptor and coreceptor antagonists may evolve the ability to bind to and utilize receptor-inhibitor complexes.

—Petropoulos CJ, Huang W, Toma J, Fransen S, Bonhoeffer S, Whitcomb JM. Resistance to HIV-1 entry inhibitors may occur by multiple molecular mechanisms. *Antiviral Therapy* 2004;9:S25. Abstract 19.

3. PATHOGENESIS, FITNESS, AND RESISTANCE

Flushing out latent HIV without rousing resting CD4s.

Eradication of HIV proved impossible with standard therapy because the virus can retreat to sundry reservoirs beyond the reach of antiretrovirals. Activating resting CD4 cells can expose latent HIV to antiretrovirals, but the activated cells become targets for new rounds of infection. Now work by David Margolis (University of Texas Southwestern Medical School, Dallas) has identified agents that may induce expression of quiescent HIV without rousing the resting cells where that virus hides.

Margolis tested three agents for their ability to induce expression of HIV from cells sampled from 10 antiretroviral-treated people with plasma loads below 50 copies/mL:

- Valproic acid, an inhibitor of histone deacetylase (which contributes to quiescence of HIV in resting CD4 cells)
- The p38 kinase inhibitor SB203580
- Interleukin 7 (IL-7), a cytokine thought capable of spurring latent HIV expression without activating T cells

Valproic acid, a drug already widely used in clinical practice, induced acetylation of integrated HIV but did not activate CD4 cells or cause new rounds of infection. At concentrations achievable in humans, valproic acid flushed HIV from resting CD4s collected from 5 of 5 aviremic individuals. Margolis recovered no HIV from IL-2-stimulated control cultures containing 24 million to 48 million CD4 cells per person. IL-7 induced outgrowth of HIV in samples from 4 of 4 people, while SB203580

accomplished the same thing (though less efficiently) in cells from 4 of 4 people. In work he did not present in detail, SB203580 did not enhance new infection of CD4 cells or activate resting CD4s.

If these strategies prove practical clinically, Margolis suggested they could shrink viral reservoirs and so perhaps contribute to more durable viral suppression.

—Margolis DM, Lehrman G, Archin NM, Ylisastigui L, Kvanl MB, Turner D, Wagner J, Wise H, Bosch RJ. Targeting reservoirs of human immunodeficiency virus infection: inducing latent viral expression without host cell activation. *Antiviral Therapy* 2004;9:S77. Abstract 68.

HIV variation and recombination high even at low viral loads.

A study of treatment-naive and treatment-experienced people found high and equivalent viral diversity in the two groups and frequent viral recombination. Using two different analytical approaches, Frank Maldarelli (HIV Drug Resistance Program, National Cancer Institute, Frederick) estimated a recombination rate between 0.5 and 3 events per genome per replication cycle. A separate study by Wei-Shau Hu, also at NCI, Frederick, reached an even higher estimate of recombination.

Comparing viral populations in 9 drug-naive people and 6 treated, viremic individuals, Maldarelli found highly diverse populations in both groups that varied little over several years. By linkage analysis he estimated a rate of 2 to 3 recombination events per genome per replication cycle. By disequilibrium analysis the estimated rate reached a still-zesty 0.5 events per genome per replication cycle. Maldarelli detected no difference between naive and treated individuals in the minimal recombination rate. Because variability proved independent of viral load over a 1000-fold range, he proposed that even low loads are laden with enough infected cells to avoid a genetic bottleneck (which would limit variability).

Maldarelli advanced the following conclusions:

- HIV-1 populations are highly diverse in both treatment-naive and -experienced people.
- Recombination in humans may be as frequent as mutation.

- These high recombination rates suggest that the number of cells infected by two or more HIV-1s may exceed 20% of the total infected pool.

Recombination rates directly affect viral susceptibility to drugs, observed Wei-Shau Hu, because separate single drug-resistant viruses can recombine to yield multidrug-resistant virus. To estimate the ability of HIV to generate viral variants, Hu and colleagues developed a flow cytometry-based assay that rates recombination according to the separation of markers lying less than 1 kb apart on the viral genome during one round of replication.

Table 2. HIV recombination rates according to length of marker separation

<i>Number of basepairs separating markers</i>	<i>Percent of maximum measurable recombination rate</i>
586	56
302	38
287	31
100	12

Hu determined that that the recombination rate of HIV-1 is proportional to the distance between markers when they lie less than 600 basepairs apart (Table 2). On the basis of these analyses, she figured that HIV-1 recombines at a rate of 11% per 100 base pairs per replication cycle. That translates into about 10 recombination events during reverse transcription of a 10-kb HIV-1 genome. Hu also found that viral accessory genes have little effect on recombination because similar recombination rates occurred in the presence or absence of *vif*, *vpr*, *vpu*, and *nef*.

—Maldarelli F, Kearney M, Palmer S, Polis M, Mican J, Stephens R, Rock D, Mellors J, Coffin J. HIV-1 populations are large, highly diverse, and characterized by frequent recombination in drug-naive and drug-resistant individuals. *Antiviral Therapy* 2004;9:S54. Abstract 45.

—Rhodes T, Nikolaitchik O, Chen J, Hu WS. High rates of HIV-1 recombination in T cells. *Antiviral Therapy* 2004;9:S53. Abstract 44.

CD8s recognize RTI-resistant virus more often during good viral control. In people with a low viral load despite reverse transcriptase inhibitor (RTI) mutations, CD8 cells recognized this mutant virus, whereas CD8 cells in people with high viral loads did so less often. Those findings,

suggested Assia Samri (Inserm U543, Paris), imply that good viral control bolsters this type of cross-recognition.

The study involved people taking at least two RTIs. Group 1 consisted of 28 people with a viral load persistently below 10,000 copies/mL and a median CD4 count of 341 cells/ μ L, while the 12 people in group 2 had a viral load above 30,000 copies/mL and a median CD4 count of 53 cells/ μ L. The median number of PI and RTI mutations stood at 7 in group 1 and 13 in group 2 ($P = 0.004$).

Recognition of wild-type RT immunodominant regions proved higher in group 1 (189 spot-forming colonies [SFC]/ 10^6 PBMCs) than in group 2 (53 SFC/ 10^6 PBMCs), though this difference lacked statistical significance ($P = 0.26$). But when Samri considered only virologic responders in both groups, recognition of wild-type RT immunodominant regions proved significantly higher in group 1 (425 SFC/ 10^6 PBMCs) than in group 2 (97 SFC/ 10^6 PBMCs) ($P = 0.04$).

The intensity of CD8 responses figured as SFC/million PBMCs proved significantly higher in group 1 than in group 2 ($P < 0.0001$). In addition, recognition of epitopes at specific RT sites differed between the two groups. CD8 cells from 5 of 10 group 1 members (50%) with a position 41 mutation recognized mutant or wild-type epitopes around RT position 41, compared with 2 of 9 (22%) in group 2. In contrast CD8s from 4 of 9 group 2 members (44%) with a position 184 mutation recognized that site, compared with 8 of 23 group 1 members (35%). HLA haplotype strongly influenced recognition of position 41 and 184 mutations. Overall, CD8s from group 2 members recognized mutated epitopes more frequently than did CD8s from group 1 members, but group 2 lost simultaneous recognition of wild-type epitopes.

—Samri A, Jeannin P, Marcelin AG, Costagliola D, Alatrakchi N, Biligui A, Agher R, Calvez V, Duvivier C, Katlama C, Autran B. CD8 T cell recognition of HIV-1 RT mutations induced by therapy with RT-inhibitors in HIV-infected patients with persistent low viral load. *Antiretroviral Therapy* 2004;9:S73. Abstract 64.

Autologous antibodies may help control virus in some with chronic infection. The ability of neutralizing antibodies to dampen viral replication in people with chronic HIV infection has not been established. But close study of 21 people enrolled in the Swiss-Spanish Intermittent Treatment Trial (SSITT) suggests that stronger antibody titers do contribute to viral control in a subset of individuals.

SSITT researchers monitored people with well-controlled viral replication through four cycles of 2-weeks-off/8-weeks-on therapy followed by a prolonged treatment break. Huldrych Günthard and coworkers scrutinized viral diversity and antibody titer in 7 people who controlled HIV while off therapy (viral load below 5000 copies/mL for at least 2 months during the prolonged break) and 14 people who did not. Günthard generated clonal HIV *env* sequences from pretreatment RNA and from rebound RNA at weeks 2, 12, 22, 32, 42, and at 1, 1.5, 2, and 2.5 years. He collected virus during the last treatment interruption and tested plasma samples for neutralizing activity against those viruses.

Mean pretreatment diversity of *env* was lower in responders than in nonresponders. During the last long treatment break, viral diversity rose in both responders and nonresponders and approached pretreatment levels. But Günthard detected adaptive evolutionary changes more often in responders, who also had higher baseline neutralizing antibody titers and bigger gains in neutralizing antibody titers over time.

Günthard concluded that positive selection coupled with higher antibody titers indicate that antibodies exerted selection pressure in responders. Together these results suggest that neutralizing antibodies help control viremia in some people with chronic infection.

—Joos B, Trkola A, Kuster H, Fischer M, Wong JK, Böni J, Leemann C, Hirschel B, Weber R, Günthard HF. Selection pressure by neutralizing antibodies results in higher adaptive mutation rates in *gp120*. *Antiviral Therapy* 2004;9S76. Abstract 66.

4. NEW RESISTANCE TECHNOLOGIES AND INTERPRETATIONS

Measuring the impact of drug history on resistance.

Standard genotyping and phenotyping tell only part of the resistance story in people with long drug histories because these tests miss small populations of resistant virus that arose during earlier treatment. Marshalling data from his Stanford HIV RT and Protease Sequence database, Robert Shafer devised a graphical model to see if treatment history can be used to flesh out a person's current resistance profile.

Shafer mined the Stanford database for 4200, 5300, and 2400 genotype-treatment correlations for protease inhibitor, nucleoside, and nonnucleoside regimens. He used those correlations to predict the probability of mutant or wild-type virus based on past treatment. The model assumes that each

drug a person ever took independently sways the probability that mutant virus lies at each key resistance site. Shafer used 70% of the dataset to build his graphical model and 30% to test the model's accuracy.

The model correctly called resistance at 77% of PI resistance positions and 76% for both nucleoside and nonnucleoside positions. Specificity measured 76% for PIs and 78% for NRTIs and NNRTIs. The high sensitivity of the model, Shafer observed, suggests that the association between mutations and drugs is highly nonrandom.

Shafer is now elaborating this model to reflect interactions among drugs and among mutations. For example, a model that takes into account correlations between mutations such as D30N and N88D, along with the lack of correlation between D30N and L90M, will likely perform better than the current model. He believes refined versions of the model may supplement genotypic and phenotypic results in people with deep treatment experience and may find a use in countries where standard resistance testing is too expensive.

—Ravela J, Raina R, Rhee SY, Schapiro JM, Shafer RW. *Probabilistic graphical models for prediction of HIV-1 drug resistance mutations based on antiretroviral treatment history. Antiviral Therapy 2004;9:S124. Abstract 110.*

Can resistance be read in a dry blood spot? The need to ship frozen plasma samples on dry ice adds greatly to the cost of clinical trials and puts viral load monitoring and resistance testing beyond the reach of most countries with runaway HIV epidemics. A dried plasma method for shipping patient samples—SampleTanker—could change that.

Robert Lloyd (Research Think Tank, Alpharetta, Georgia) spelled out results of a study that compared the dried plasma spot method with standard frozen samples. Researchers randomly picked archived frozen plasma samples and readied duplicate 1-mL frozen and dried specimens for shipping. They prepared the dried specimens by adding 1 mL of plasma to the SampleTanker matrix, air drying the samples, and shipping them in individual sealed tubes exposed to ambient temperatures.

At the shipment's receiving end, technicians successfully measured viral loads and determined genotypes from all frozen and dried samples. Reconstitution of the dried 1-mL samples yielded an average 0.998 mL of plasma. Viral loads were consistently lower with SampleTanker than with other assays, by an average 0.36 log copies/mL. The study

showed high concordance between matched dried and frozen samples in detecting resistance-linked mutations. This concordance proved consistent regardless of viral load, storage time, or shipment conditions before genotyping. Genotype accuracy and reproducibility with the dried method compared well with data published in the TruGene genotype product insert.

Lloyd concluded that SampleTanker could significantly lower plasma sample transportation costs for clinical use and trials regardless of site.

—Lloyd RM Jr, Burns DA, Thompson AM, Mathis RL, Holodniy M, Huang JT, De La Rosa A, Yen-Lieberman B, Armstrong W, Taege A, McClernon DR, Feorino PM. *Comparison of HIV-1 viral load and resistance genotyping between frozen plasma and a novel dried plasma transportation matrix. Antiviral Therapy 2004;9:S135. Abstract 121.*

Assay simultaneously spots HIV and HCV resistance.

About 30% of HIV-infected people in the US and Europe are coinfecting with hepatitis C virus (HCV), which has a modest response to interferon plus ribavirin in coinfecting people. Although reasons for this lackluster response have not been nailed down, changes in two HCV genes—NS5A and NS5B—have been tied to resistance to interferon and ribavirin respectively. In an attempt to streamline the study of resistance in coinfecting people, Michael Kozal (Yale University, New Haven) devised an assay that simultaneously probes for HCV and HIV resistance.

Kozal's microarray, built by Affymetrix, embraces the consensus sequences for HCV genotypes 1a and 1b NS5A, NS5B, and NS3 genes and about 1040 bases of the HIV-1 clade B *pol* gene covering reverse transcriptase and protease. Alternate arrays for known drug resistance mutations allow for quantification of mutant and wild-type mixtures.

Using clones and samples from coinfecting patients, Kozal and colleagues hybridized 6 Kb of RT-PCR products from HIV-1 *pol* and HCV clade 1a NS5A and NS5B genes to the microarray. The assay's base call accuracy measured 99% in HIV-1 *pol* and 98% for the HCV genes. The assay can detect minority variants representing as little as 1% of the viral population. The researchers suggested this new tool will simplify study of genetic triggers to HCV resistance and elucidate the effect of interferon and ribavirin on HIV genes in coinfecting people.

—Kozal M, Chiarella J. *Development of a DNA microarray to detect HCV and HIV drug resistance. Antiviral Therapy 2004;9:S136. Abstract 122.*

HIV-1 subtype will affect genotype advice. Algorithms that interpret resistance from viral genotype vary slightly in treatment recommendations because of inclusion or exclusion of some substitutions and subtle differences in weighing the impact of mutation sets. Those differences may grow as genotyping becomes more common for people infected with virus other than HIV-1 subtype B, according to results of an international study reported by Joke Snoeck (Rega Institute, Leuven).

Snoeck and colleagues rated concordance in predicting viral susceptibility and treatment response with four standard interpretation systems—Rega 5.5, the Stanford HIVDB-03/02, ANRS (09/02), and the Visible Genetics/Bayer VGI 6.0. Overall concordance between algorithms proved high—94% for nonnucleosides, 76% for protease inhibitors, and 75% for nucleosides. But interpretive discordance varied from one viral subtype to the next. For example, treatment-naive people infected with subtype F produced the most discordant results for all PIs because of a high rate of mutations at positions 10 and 20. Treatment-naive people with subtype C virus had more discordant results for ritonavir and indinavir, traced to changes at protease positions 10 and 36, and for ddC, traced to reverse transcriptase codon 69.

Significantly more discordances arose in treatment-experienced people than in naive individuals ($P < 0.0001$). The most striking difference between experienced and naive people was a high discordance rate for efavirenz, attributable to single mutations at reverse transcriptase positions 190 and 181.

Although the algorithms generally agreed well, Snoeck and coworkers concluded, they turned up several examples of subtype-dependent discordance. Whether response to antiretrovirals varies by subtype remains unknown, they added, but the advice clinicians get can vary from subtype to subtype depending on the algorithm used. (Another Workshop study found differing resistance patterns in nevirapine-exposed women with subtype A versus D. See “Differing NNRTI resistance patterns” under “5. Clinical Implications.”)

—Snoeck J, Kantor R, Shafer RW, Van Laethem K, Deforche K, Carvalho AP, Wynhoven B, Soares MA, Cane P, Clarke J, Pillay C, Sirivichayakul S, Ariyoshi K, Holguin A, Rudich H, Rodrigues R, Bouzas MB, Brun-Vézinet F, Reid C, Cahn P, Brigido LF,

Grossman Z, Soriano V, Sugiura W, Phanuphak P, Morris L, Weber J, Pillay D, Tanuri A, Harrigan PR, Camacho R, Schapiro JM, Katzenstein D, Vandamme AM. *Discordances between interpretation algorithms for genotypic resistance to protease and reverse transcriptase inhibitors are subtype dependent. Antiviral Therapy 2004;9:S126. Abstract 112.*

Expert advice varies with the resistance test used. Does it help to consider both genotype and phenotype rather than either test alone when planning a rescue regimen? Trying to answer that question, Andrew Zolopa (Stanford University) devised a devilish exercise that asked 13 resistance mavens to recommend new drugs for 5 people based on genotype plus phenotype (GT/PT) or the same GT or PT alone. To make the test even tougher, Zolopa gave the experts no other information about each patient, and each sample had at least one source of GT-vs-PT discordance resulting from the presence of mutant/wild-type mixtures, suppressive effects of mutations, or cross-resistance not accounted for by genotype.

A three-way comparison of antiretroviral choices based on GT, PT, or GT/PT showed differences for all 15 drugs chosen by at least one expert. Zolopa figured the degree of difference for each drug as the percentage of difference between frequency of selecting that drug based on one type of test versus another compared with maximum possible differences. By that criterion, he charted degrees of difference ranging from 100% for nevirapine and atazanavir to 13% for 3TC.

The median difference measured 47%; in other words, nearly half the time that experts picked a drug on the basis of one test result, they did not choose that drug on the basis of at least one other test. Differences proved larger for GT versus PT and GT versus GT/PT than for PT versus GT/PT, especially for tenofovir and somewhat less for abacavir, amprenavir, indinavir, and saquinavir. PT and GT/PT results favored picking ddI, abacavir, and efavirenz more than GT alone. GT favored selecting AZT, tenofovir, and saquinavir more than PT or GT/PT. GT/PT promoted the choice of AZT, 3TC, or lopinavir/ritonavir more than PT.

The study did not determine whether picking different regimens for these 5 people would have an impact on clinical outcome. But Zolopa believes the differing picks with different assays suggest a clinical value for combined GT/PT results, at least in some circumstances.

—Zolopa AR, Bates M, Parkin N. Experts select different antiretroviral drug regimens when presented with resistance data in the form of genotype, phenotype, or combined genotype plus phenotype. *Antiviral Therapy* 2004;9:S130. Abstract 116.

5. EPIDEMIOLOGY

New method shows spreading triple-class resistance in UK. Studies in different countries and regions disagree on whether multidrug-resistant virus has become more prevalent in recent years. The method used to measure resistance can contribute to these differing results, according to findings by Deenan Pillay (University College London). He logged resistance trends in the UK Collaborative HIV Cohort Study by two methods:

- *Method A:* Genotypic testing done within a given calendar period without reference to previous tests in cohort members
- *Method B:* Genotypic testing plus calculation of archived resistant virus by reference to all previous tests, using a denominator of all people ever treated and still alive at selected time points.

The analysis involved over 10,000 people who began antiretroviral therapy and were alive after 1998. Method A suggested that triple-class resistance remained flat in the UK from 1999 through 2002, whereas method B indicated a lower but rising rate (Table 3).

Table 3. UK triple-class resistance rates figured by two methods

Year	Method A (%)	Method B (%)
1999	16.3	4.6
2000	16.8	6.1
2001	17.0	6.3
2002	13.6	7.1

The absolute number of tests showing triple-class resistance by method A climbed from 43 in 1998 to 191 in 1999 but then changed little through 2002. Method B counted 118 cases of triple-class resistance in 1998, 377 in 1999, 590 in 2000, 718 in 2001, and 816 in 2002.

Method B probably underestimates the rate of triple-class resistance, Pillay noted, because resistance testing

was done at only 16% of drug switches, when detection of resistant virus would be more likely. He characterized the study as exploratory work based on a rough analysis using the IAS-USA table of resistance mutations. Although the numbers showed a growing overall burden of resistance in the UK, multidrug resistance apparently still affects a low proportion of all treated people.

—Pillay D, Green H, Gazzard B, Pozniak A, Matthias R, Johnson M, Churchill D, Fisher M, Hill T, Geretti AM, Clarke J, Cane P, Loveday D, Scullard G, Easterbrook P, Porter K, Williams I, Gilson R, Sabin C, Phillips A, Dunn D. Estimating resistance in drug experienced patients in the UK. *Antiviral Therapy* 2004;9:S88. Abstract 77.

One in five in US gets non-HAART regimens. A CDC study of people taking antiretrovirals in four US cities found that 19% got non-HAART regimens in 2001-2003. The overall resistance rate in the 774-person study reached a prodigious 79%.

Amanda Smith tracked resistance trends in 774 people who had at least one genotype in Los Angeles, New Orleans, New York, or Seattle from November 2001 through October 2003. Defining HAART as at least 3 drugs from 2 classes or Trizivir (AZT, 3TC, abacavir), she found that 149 (19%) got a non-HAART regimen. In the whole group 613 (79%) had one or more resistance mutations, 303 (39%) had mutations to 2 antiretroviral classes, and 141 (18%) had resistance to 3 classes. Resistance to nucleosides proved most prevalent (71%), though resistance to nonnucleosides (44%) and protease inhibitors (40%) was not scant.

—Smith AJ, Wang H, Bennett D, Teshale E, Buskin S, Morse A, Wohl A, Swerdlow D, Sullivan P, Wolfe M. Prevalence of mutations associated with antiretroviral drug resistance in a cohort of treated individuals in four US cities. *Antiviral Therapy* 2004;9:S112. Abstract 101.

Transmission of NNRTI-resistant virus linked to higher viral loads. A study of antiretroviral-naive people with primary HIV infection found higher viral loads in those infected with NNRTI-resistant virus than in those infected with wild-type virus. But viral loads were lower in people infected with virus resistant to nucleosides or PIs than in people infected with wild-type virus.

Susan Little (University of California, San Diego) and colleagues from other institutions charted viral loads and

resistance patterns in 340 recently infected individuals, while measuring replicative capacity of infecting virus in 53. Nearly all cohort members (94%) were men, and 68% were white non-Hispanics.

Compared with baseline viral loads in people with nonmutant virus, baseline loads were significantly lower in those infected with virus that had high-level genotypic resistance to one or more nucleosides ($n = 35$, $-0.7 \log_{10}$ copies/mL, $P < 0.001$) or PIs ($n = 24$, $-0.5 \log_{10}$ copies/mL, $P < 0.05$). Those results support findings that resistant virus has a lower replicative capacity than nonresistant virus. But infection with NNRTI-resistant virus did not fit this replication-capacity model. Little found significantly *higher* baseline viral loads in people infected with NNRTI-resistant virus ($n = 53$, $+0.6 \log_{10}$ copies/mL, $P < 0.001$) than in those infected with nonmutant virus.

After 1 year of follow-up, viral loads were $0.5 \log_{10}$ copies/mL higher in people with NNRTI-resistant virus than in those with wild-type virus. In contrast loads were $0.8 \log_{10}$ copies/mL lower in the combined group with virus resistant to NRTIs or PIs than in those with wild-type virus. In the subset of 53 people, replication capacity had no effect on viral set point. But Little noted the over-representation of people taking nonnucleosides in this subset.

Why transmission of NNRTI-resistant virus results in higher viral loads remains unknown, Little observed, but higher replicative capacity of that virus is not the answer. Viral loads in a large cohort of recently infected people may appear comparable in those with resistant and nonresistant virus, she suggested, because the positive viral load effect of NNRTI-resistant virus offsets the negative effect of NRTI- and PI-resistant virus.

—Little SJ, Grant RM, Daar ES, Markowitz M, Hecht FM, Johnson V, Allen T, Frenkel LM, Benson C, Routy JP, Conway B, Sun X, Richman DD, Frost SDW. Transmitted NNRTI drug resistance is associated with higher steady state viral load measures in untreated subjects with primary HIV infection. *Antiviral Therapy* 2004;9:S58. Abstract 49.

Assay spots “hidden” mutations in untreated people. A real-time polymerase chain reaction (PCR) point mutation assay spotted hallmark nonnucleoside and 3TC mutations in samples from people in whom standard genotyping saw only wild-type virus. Jeffrey Johnson (Centers for Disease Control, Atlanta) suggested that such assays could prove useful in screening treatment-naive people for drug-resistant virus.

In tests of viral specimens known to contain no mutations or to carry the K103N nonnucleoside mutant or the M184V mutant resistant to 3TC, the real-time PCR assay proved highly sensitive and specific. Johnson and colleagues calculated that it can spot K103N mutants making up only 0.4% of a viral population and M184V mutants making up only 2%.

They used the PCR assay to search for virus bearing K103N or M184V in untreated people who had other reverse transcriptase mutations detected by conventional assays. The assay aimed at K103N detected 4 positive samples (2.4%) in 165 isolates that appeared to be free of that mutation on standard genotyping. The M184V PCR assay turned up 3 positive samples (1.7%) in 173 isolates rated free of that mutation by conventional testing.

—Johnson JA, Li JF, Bennett D, Cong M, Spira T, Shafer RW, Gleeson T, Sandstrom P, Heneine W. Real-time PCR assays identify transmitted drug resistant HIV-1 previously undetected by conventional nucleotide sequencing. *Antiviral Therapy* 2004;9:S87. Abstract 76.

Does resistance drive mortality in today’s treatment era?

Although many intuit that resistance ultimately kills people with HIV despite the potent regimens available today, two studies addressing that question reached different answers. Analyzing resistance rates and mortality trends in antiretroviral-treated treated British Columbians, Richard Harrigan (BC Centre for Excellence in HIV/AIDS, Vancouver) determined that a large majority of deaths do not reflect exhaustion of treatment options because of drug-resistant virus. But Mauro Zaccarelli (National Institute for Infectious Diseases Lazzaro Spallanzani, Rome) found resistance to 3 antiretroviral classes a reliable harbinger of progression or death.

The 2 British Columbian populations consisted of 637 HIV-infected people who died from July 1997 through December 2001 and 1220 people with virologic failure but alive at the end of 2001. In both groups Harrigan based his resistance calculation on the last on-therapy plasma sample.

Of the 554 nonaccidental deaths recorded, 58 (10.5%) involved people who had no antiretroviral experience. Most of the remaining deaths occurred in 99 people (17.9%) with a short duration of treatment (median 2 months) or in 147 (26.5%) with a viral load below 500 copies/mL (and thus a low likelihood of resistance). Death proved significantly more likely among people who first received monotherapy

or dual therapy than among those who started with a triple regimen ($P < 0.0001$).

Using IAS-USA mutations lists, Harrigan determined that 56 (10%) in the mortality group had single-class resistance while 89 (14%) had double-class resistance. In contrast, among the 1220 people alive with virologic failure, 75% had single-class resistance, 42% double-class resistance, and 11% triple-class resistance. No one in this group who started treatment with triple-class therapy had triple-class resistance.

The low prevalence of broad resistance among people who died, Harrigan proposed, indicates that resistance was not the main reason for death in this population.

Mauro Zaccarelli and colleagues also used IAS-USA resistance tables to gauge resistance in 623 antiretroviral-treated people tracked for death from any cause or an AIDS event or death over a median of 574 days (interquartile range [IQR] 373 to 875 days). They found that mounting resistance inflated the risk of progression or death.

The cohort included 306 people (49.1%) without multidrug resistance to any class, 215 (34.5%) with multidrug resistance to 1 class, 78 (12.5%) with multidrug resistance to 2 classes, and 24 (3.9%) with multidrug resistance to three classes. The group's median baseline CD4 count stood at 302 cells/ μ L (IQR 154 to 464 cells/ μ L) and the median viral load at 4.39 logs (IQR 3.84 to 4.94 logs). The group's charts showed a median of 3 regimen failures (IQR 1 to 4).

A Cox model plucked out several independent predictors of progression or death:

- Prior AIDS: hazard ratio (HR) 1.91, $P = 0.028$
- Resistance to 3 antiretroviral classes: HR 3.34, $P = 0.008$
- CD4 count as a time-dependent variable: HR 0.79, $P < 0.001$
- Lopinavir in salvage regimen: HR 0.53, $P = 0.051$

The probability of survival or remaining free from a new AIDS diagnosis fell with the loss of each additional antiretroviral class to resistance (Table 4).

—Brumme ZL, Rescky M, Wynhoven B, Dong W, Chan K, Yip B, Sattha B, Montaner J, Hogg R, Harrigan PR. HIV drug resistance and treatment failure: contrasting mortality and viral load endpoints. *Antiviral Therapy* 2004;9:S95. Abstract 84.

—Zaccarelli M, Tozzi V, Lorenzini P, Forbici F, Trotta MP, Visco-Comandini U, Gori C, Boumis E, Bellocchi MC, Bellagamba R, D'Arrigo R, Liuzzi G, Narciso P, Perno CF, Antinori A. Does drug class multi-resistance affect survival? Analysis from a cohort of HIV patients who experienced treatment failure. *Antiretroviral Therapy* 2004;9:S155. Abstract 139.

Table 4. Probability of survival or remaining AIDS free according to class resistance

	Multidrug resistance to:			
	0 classes	1 class	2 classes	3 classes
Survival at 36 months (%)	91	88	86	73
Free of AIDS or death at 36 months (%)	84	83	81	64

Genetic barrier to resistance similar with B and non-B subtypes. Analysis of reverse transcriptase (RT) and protease gene sequences from European CATCH study participants—including more than 600 people with HIV-1 subtypes other than B—found no major differences between subtypes in the calculated genetic barrier to resistance. In fact, some evidence hinted at a higher barrier to resistance with non-B subtypes.

David van de Vijver (University Medical Center Utrecht) and colleagues across Europe and in Israel scrutinized protease and RT sequences in virus from untreated people with HIV-1 subtypes A, B, C, D, F, G, J, CRF01-AE, and CRF02-AG. They defined genetic barrier as the number of nucleotide changes required for evolution to drug resistance mutations. No substantial differences emerged between subtype B virus and any of the other subtypes studied, although the analysis did turn up some subtle variations in certain subtypes:

- Almost all subtype G sequences had isoleucine at protease position 82, rather than valine as in other subtypes, but that difference would have no impact on genetic barrier.
- Certain subtypes had evidence of a higher genetic barrier in RT, for example:
 - 72% of subtype Gs would require two nucleotide changes at position 108 before virus became resistant
 - 63% of subtype Gs would require two changes at position 118
 - 83% of subtype Fs would require three changes at position 151
 - 43% of subtype Fs, 63% of subtype Gs, and 72% of CRF02-AGs would require two changes at position 210

—van de Vijver DAMC, Wensing AMJ, Angarano G, et al. The calculated genetic barrier for drug resistance mutations in six different non-B subtypes and two CRF's in a large European dataset is largely similar to subtype B. *Antiviral Therapy* 2004; 9:S98. Abstract 87.

6. CLINICAL IMPLICATIONS OF RESISTANCE

Standardized phenotypic clinical cutoffs proposed for antiretrovirals. Analyzing virologic results from 13,000 people in 7 clinical trials and 2 cohorts, Bart Winters (VircoLab) and clinician colleagues proposed clinical cutoffs that link virologic response with viral susceptibility to current antiretrovirals. Winters and coworkers believe the results—though preliminary—suggest that clinical cutoffs determined in such a consistent manner are applicable to diverse patient populations.

Winters modeled virologic response as a function of baseline viral susceptibility (fold change in 50% inhibitory concentration, or IC₅₀, compared with wild-type virus) predicted by VirtualPhenotype analysis of viral genotype. Models constructed using change in viral load from baseline and treatment response rate included phenotypic susceptibility scores of the regimen taken, baseline viral

load, and fold change in susceptibility. The analysis determined the following fold change values (and 95% confidence intervals) associated with a 20% and an 80% diminution in response at 8 weeks compared with maximal response (Table 5).

Winters noted that although the magnitude of virologic responses for individual patients is affected by variables such as phenotypic susceptibility score, baseline viral load, and treatment history, those variables do not affect fold change values associated with percentage diminution in response. The derived clinical cutoffs are specific for the VirtualPhenotype.

—Bachelet LT, Winters B, Nauwelaers D, Rinehart A, McGregor M, Harrigan R, Perez-Elias M, Miller M, Emery S, van Leth F, Robinson P, Baxter JD, Pozniak A. Estimation of phenotypic clinical cutoffs for VirtualPhenotype through meta analyses of clinical trial and cohort data. *Antiviral Therapy* 2004;9:S154. Abstract 138.

Nevirapine resistance tied to drug levels in mother. Women in Côte d'Ivoire who took single-dose nevirapine to prevent mother-to-child transmission of HIV were more likely to have nevirapine-resistant virus 48 hours postpartum if they

Table 5. Clinical cutoffs for diminution in 8-week response to a rescue regimen

Drug	VirtualPhenotype predicted FC of wild-type isolates	Fold change cutoffs for	
		20% diminution versus maximal response (95% CI)	80% diminution versus maximal response (95% CI)
AZT	0.8	1.8 (1.5 to 2.5)	17 (10 to 25)
3TC	0.8	1.1 (1.1 to 1.2)	2.6 (1.9 to 4.6)
d4T	0.7	1.3 (1.2 to 1.4)	3.4 (3.1 to 3.6)
βddI (extended release)	0.6	1.3 (1.2 to 1.9)	3.6 (2.8 to 4.9)
Abacavir	0.6	1.6 (1.1 to 2.6)	5.8 (1.7 to 7.4)
Tenofovir	0.8	1.2 (1.1 to 1.5)	2.5 (1.7 to 3.8)
Indinavir	0.7	1.2 (1.1 to 1.9)	3.4 (1.9 to 16.4)
Indinavir/r	0.7	3.5 (1.1 to 8.4)	25 (1.8 to 31)
Amprenavir	0.6	1.2 (1.1 to 2.4)	3.4 (1.7 to 10.2)
Amprenavir/r	0.6	1.5 (1.2 to 2.6)	6.8 (3.6 to 10.5)
Nelfinavir	0.9	1.1 (1.1 to 1.3)	2.2 (1.7 to 5.3)
Saquinavir	0.6	1.1 (1.1 to 2.1)	2.0 (1.7 to 18)
Saquinavir/r	0.6	1.6 (1.3 to 4.8)	12.3 (5.8 to 27)
Lopinavir/r	0.8	6.9 (2.1 to 17.4)	56 (29 to 67)

FC = fold change; r = ritonavir boost.

had higher nevirapine blood levels. Follow-up of infected infants with nevirapine-resistant virus suggests that virus may stay archived for at least 1 year.

The study reported by Marie-Laure Chaix (CHU Necker, Paris) involved 74 women who began AZT at or after 36 weeks of gestation, then took 600 mg of AZT and 200 mg of nevirapine just before labor. Neonates received AZT for 1 week after birth and a single 2-mg/kg dose of nevirapine syrup on day 2 or 3. The transmission rate at week 6 measured 6.4%.

Twenty-one of 63 women (33%) had nevirapine-associated resistance mutations (usually K103N) detectable in plasma 4 weeks after delivery. Among 20 of those women who had genotypic analysis of peripheral blood mononuclear cell (PBMC) DNA, 15 (75%) had resistant virus. None of 3 women who had PBMC sampling 12 months after delivery had nevirapine resistance mutations detectable by reverse transcriptase sequencing.

The median nevirapine plasma concentration 48 hours after delivery measured 851 ng/mL among mothers with nevirapine-resistant virus and 598 ng/mL among those without resistant virus ($P = 0.014$), though concentration ranges of the two groups overlapped widely. A multivariate analysis isolated two factors that independently predicted nevirapine resistance mutations in mothers: A higher viral load made resistance 4 times more likely ($P = 0.012$), and a higher nevirapine concentration on day 2 raised the resistance risk 1.2 times ($P = 0.031$).

Six of 26 infected infants (23%) had one or more nevirapine resistance mutations at week 4. In two infants whose PBMCs were tested, one had nevirapine resistance 3 months after birth and one had resistance 12 months after birth.

—Chaix ML, Ekouevi DK, Peytavin G, Rouet F, Bequet L, Montcho C, Vihou I, Fassinou P, Leroy V, Dabis F, Rouzioux C. Persistence of NVP-resistant virus and pharmacokinetic analysis in women who received intrapartum nevirapine associated to a short course of zidovudine to prevent perinatal HIV-1 transmission: the Ditrane Plus ANRS 1201/02 Study, Abidjan, Côte d'Ivoire. *Antiviral Therapy* 2004;9:S176. Abstract 160.

Differing NNRTI resistance patterns with different HIV-1 subtypes. A study of Ugandan women who took single-dose nevirapine to prevent mother-to-child transmission of HIV-1 found that postpartum resistance patterns depended on whether they were infected with subtype A or subtype D.

Susan Eshleman (Johns Hopkins Medical Institutions, Baltimore) and coworkers from other sites tracked resistance mutations in 147 women with subtype A virus and 98 with subtype D both 7 days postpartum and 6 to 8 weeks afterwards. Women in the two groups had similar resistance mutation rates at day 7, but at 6 to 8 weeks 35 (36%) of those with subtype D had more mutations compared with 28 (19%) with subtype A. Eshleman pinpointed two factors that contributed to the higher resistance rate in subtype D-infected women (odds ratio 2.52, 95% confidence interval 1.14 to 5.59) at 6 to 8 weeks:

- The Y181C mutation faded from detection at a significantly greater rate in women with subtype A (odds ratio 3.06, 95% CI 1.04 to 8.90).
- The K103N mutation emerged at a greater rate in women with subtype D, although that difference lacked statistical significance (odds ratio 1.74, 95% CI 0.62 to 4.87).

The researchers detected shifts in other resistance mutations, but too few women had those mutations to allow statistical comparisons.

The results suggest that HIV-1 subtype can affect the emergence or fading of resistance mutations after exposure to antiretroviral drugs.

(Another Workshop study found evidence that interpretation of genotypic results can change from algorithm to algorithm in people infected with different HIV-1 subtypes. See “HIV-1 subtype will affect genotype advice” under “4. New Resistance Technologies.”)

—Eshleman SH, Wang J, Guay LA, Cunningham SP, Mwatha A, Brown ER, Musoke P, Mmro F, Jackson JB. Distinct patterns of selection and fading of K103N and Y181C are seen in women with subtype A vs. D HIV-1 after single dose nevirapine: HIVNET 012. *Antiviral Therapy* 2004;9:S59. Abstract 50.

Two-week responses to tipranavir/ritonavir versus other boosted PIs. A randomized trial of people with a history of 2 or more PI failures found a better 2-week response to tipranavir/ritonavir than to ritonavir-boosted amprenavir, saquinavir, or lopinavir. People with either 3 or 4 primary protease mutations responded better to tipranavir/ritonavir than to the other boosted PIs.

Douglas Mayers (Boehringer Ingelheim Pharmaceuticals) and colleagues in the US and the Netherlands randomized

people with multiple protease mutations at codons 33, 82, 84, or 90 to an optimized background regimen plus twice-daily tipranavir/ritonavir (500/100 mg, $n = 63$), lopinavir/ritonavir (400/100 mg, $n = 79$), amprenavir/ritonavir (600/100 mg, $n = 74$), or saquinavir/ritonavir (1000/100 mg, $n = 75$) for 2 weeks. After 2 weeks everyone not already taking tipranavir added tipranavir/ritonavir (500/100 mg) twice daily.

Baseline population sequencing showed that 83% of study participants had 3 mutations at positions 33, 82, 84, or 90, while 17% had mutations at all four positions. The study group had a median of 6 PI mutations, 6 NRTI mutations, and 2 NNRTI mutations; 19% had already taken enfuvirtide (T-20). Baseline phenotyping showed a 4.7-fold change in IC_{50} for tipranavir and IC_{50} s ranging from 41 to 361 for other PIs.

Median viral load reductions at week 2 proved best in the tipranavir group:

- 1.15 logs with tipranavir/ritonavir
- 0.35 log with lopinavir/ritonavir
- 0.29 log with saquinavir/ritonavir
- 0.21 log with amprenavir/ritonavir

Among people with 3 mutations at the predetermined sites, proportions who attained at least a 1-log drop in viral load at week 2 were 61% with tipranavir, 33% with lopinavir, and 25% with saquinavir or amprenavir. Among people with all 4 mutations at baseline, proportions achieving at least a 1-log viral load decline were 42% with tipranavir, 20% with lopinavir, 18% with saquinavir, and 13% with amprenavir.

After study week 4, when everyone had taken tipranavir/ritonavir for at least 2 weeks, viral loads rebounded toward baseline in every treatment arm. Mayers attributed this failure to difficulties in constructing a good background regimen for people with such deep treatment experience. In phase 3 studies, he noted, heavily experienced people who respond best to tipranavir/ritonavir are those who can add T-20 at the same time.

—Mayers D, Leith J, Valdez H, Boucher CA, Schapiro J, Baxter J, McCallister S, Kohlbrenner VM, Scherer J, Hall DB. Impact of 3 or 4 protease mutations at codons 33, 82, 84 and 90 on 2 week virologic responses to tipranavir, lopinavir, amprenavir and saquinavir all boosted by ritonavir in phase 2B trial BI 1182.51. *Antiviral Therapy* 2004;9:S163. Abstract 147.

First report of isolated resistance to lopinavir/ritonavir.

Mutations conferring resistance to lopinavir/ritonavir typically do not emerge during failure of a lopinavir/ritonavir regimen in people with no earlier PI experience. Steven Deeks (San Francisco General Hospital) chronicled one such case at the Workshop.

A PI-naive man taking AZT, 3TC, tenofovir, and efavirenz replaced the nonnucleoside with lopinavir/ritonavir, then decided to abandon the other drugs in his regimen. Over the ensuing year adherence to lopinavir was probably spotty since pharmacy records show that he did not refill his prescription one month. When his viral load rebounded above 14,000 copies/mL, genotyping disclosed a V82A change, which was replaced by V32I, M46M/I, and I47A. This virus had high-level resistance to lopinavir with a 38-fold change in 50% inhibitory concentration (IC_{50}) compared with wild-type virus.

The mutant virus proved hypersusceptible to saquinavir (0.16-fold change in IC_{50}) while retaining susceptibility to other protease inhibitors (highest fold change in IC_{50} at 2.7 for indinavir). Renewed treatment with a saquinavir/ritonavir regimen pushed his load below 50 copies/mL once more. A look through ViroLogic's database turned up 53 isolates with the I47A mutation, 40 of which had the V32I change, and 14 with only V32I and I47A. Replication capacity measured a paltry 13.5% in 27 of the I47A isolates tested, and only 3.8% in 3 V32I/I47A mutants.

Deeks concluded that although primary resistance to lopinavir/ritonavir remains exceedingly rare in the clinic, it can happen, and the preferred route may involve V31I and I47A.

—Parkin NT, Petropoulos CJ, Chappey C, Friend J, Liegler T, Martin JN, Deeks SG. Isolated lopinavir resistance after virologic rebound of a lopinavir/ritonavir-based regimen. *Antiviral Therapy* 2004;9:S79. Abstract 70.

Early failure with efavirenz, ddI, and tenofovir.

A trial that randomized treatment-naive people to once-daily efavirenz, ddI, and tenofovir or the same regimen plus lopinavir/ritonavir found a high rate of virologic failure in the 3-drug group along with an unusual resistance pattern. The results came as a surprise since efavirenz has proved potent when combined with other nucleosides in previously untreated people.

Daniel Podzamczar (Universitari de Bellvitge, Barcelona) and coworkers at other sites assigned 17 people to the 3-drug arm and 19 to the 4-drug arm. (Recruitment ended earlier than planned when the triple-drug regimen began failing.) People in the 3-drug group started treatment with a median CD4 count of 212 cells/ μ L (range 7 to 490 cells/ μ L) and a median viral load of 207,372 copies/mL (range 30,166 to >500,000 copies/mL). Early failures in the 3-drug group led to an unplanned interim analysis involving 26 people who had completed 3 months of therapy.

At that point Podzamczar and coworkers counted 6 virologic failure among 14 people on the 3-drug regimen (43%) versus none among 12 people on the four-drug regimen ($P = 0.017$). (They defined virologic failure as failure to achieve a 2-log drop in viral load by treatment month 3, a 1-log viral load rebound.) Comparing the 6 treatment failures with the 8 responders in the 3-drug arm, they found more advanced disease in the failing group (Table 6).

Table 6. Early failure versus response with ddl, tenofovir, and efavirenz

Baseline traits	Failure (n = 6)	Response (n = 8)	P
Median CD4 count (cells/ μ L)	73.5	217	0.011
CD4 <200 cells/ μ L	6 (100%)	3 (37.5%)	0.031
CD4 <100 cells/ μ L	3 (50%)	0	0.055
Median viral load (copies/mL)	317,474	118,611	0.044
Prior AIDS or B3 disease	6 (100%)	0	<0.001

The only relevant baseline mutations were T69D/N in 1 person and T69S in another. Genotyping at virologic failure detected G190S/E alone or with K103N in 5 of 6 people. These efavirenz-linked mutations appeared as early as day 14 of treatment and as late as month 3. Six people had the L74V/I mutation at failure, and 2 had K65R.

What went wrong? Attendees suggested that selection of mutations at the 65 and 74 sites may reflect counterselection of thymidine analog mutations (TAMs) by efavirenz, because TAM mutants are hypersensitive to this nonnucleoside. Earlier work suggested that G190E mutant

virus promotes evolution of L74V as a compensatory mutation. Perhaps this once-daily combination is too frail in people with high viral loads because replication continues long enough after therapy begins to select a mere 2 regimen-killing mutations—G190S and L74V.

—Podzamczar D, Ferrer E, Gatell JM, Niubo J, Dalmau D, Leon A, Knobel H, Iniguez D, Ruiz I. Early virologic failure and occurrence of resistance in naive patients receiving tenofovir, didanosine and efavirenz. *Antiviral Therapy* 2004;9:172. Abstract 156.

New or archived mutations emerge during 18% of STIs.

A study of 35 people enrolled in four structured treatment interruption (STI) trials charted the emergence or new or archived resistance mutations in 9 of them (26%) during 20 of 112 STI cycles (18%). Mutations conferring resistance to 3TC or nonnucleosides proved the most common.

Testing for mutations before these people started antiretrovirals, before they started their STIs, and during the STIs, Mireia Arnedo (University of Barcelona) found new mutations during 5 STI cycles and re-emerging archived mutations during 4 cycles. The most common mutations were those linked to 3TC (in 50% taking 3TC) or nonnucleosides (in 23% taking a nonnucleoside), whereas no protease mutations arose (Table 7).

Table 7. Emergence of new or archived mutations during an STI cycle

Conferring resistance to:	New mutations	Archived mutations
Nonnucleosides	27%	0%
3TC	12%	24%
Nucleosides other than 3TC	3%	3%
Protease inhibitors	0%	0%

Arnedo found that most mutations appeared during the first STI cycle; the mutation rate did not rise significantly over successive cycles. VirtualPhenotype correlated well with genotype.

—Arnedo M, Garcia F, Gil C, Castro P, Blanco JL, Miró JM, Pumarola T, Gatell JM. Risk of developing selected de novo resistance mutations during structured therapy interruption (STI) in chronic HIV-1 infection. *Antiviral Therapy* 2004;9:S158. Abstract 142.

Steady accretion of mutations in clinical practice. A survey of 4487 people starting 3 or more antiretrovirals charted a virologic failure rate of 42% within 6 years and one or more major resistance mutations in 27%.

Andrew Phillips (Royal Free and University College Medical School, London) scrutinized virologic and genotypic records of 4487 people starting at least a triple regimen from January 1996 at 6 clinics in London and Brighton; 56% started with an NNRTI and 41% with a PI. Median follow-up time stretched to 183 weeks. Defining failure as consecutive viral loads above 1000 copies/mL after 24 weeks (not counting treatment interruptions) or one load above 1000 copies/mL and at least one new drug, he graphed an overall failure rate of 26% and a 42% rate among people tracked for 6 years.

At 6 years 27% had at least one resistance mutation, 18% had mutations to at least 2 antiretroviral classes, and 3.5% had triple-class resistance. Rates of all nucleoside mutations rose from 8% after 2 years of follow-up, to 17% after 4, and to 23% after 6 (4%, 9%, and 13% for all thymidine analog mutations), while the prevalence of PI mutations climbed from 3% at 2 years, to 7% at 4 years, and to 9% at 6 years. Respective rates of nonnucleoside mutations measured 6%, 12%, and 16%.

A multivariate analysis linked several factors to lower or higher risks of at least one resistance mutation emerging during treatment at the following relative hazards (RH):

- Baseline viral load <10,000 copies/mL (RH 0.86) or 10,000 to 99,999 copies/mL (RH 0.64) versus >100,000 copies/mL ($P < 0.001$)
- AIDS diagnosis at baseline versus no AIDS (RH 1.33, $P = 0.003$)
- PI therapy with no NNRTI versus NNRTI therapy with no PI (RH 1.18, $P < 0.005$)
- Four or more versus 3 drugs in the initial regimen (RH 0.71, $P = 0.03$)

Phillips believes these resistance rates are underestimates because not all cohort members had resistance tests at virologic failure (when such tests most readily detect resistant virus) and because standard resistance assays do not detect minority viral variants. The large and ever-increasing proportion of people with resistant virus suggests to these researchers that resistance is not a phenomenon unique to subgroups who have a hard time with adherence.

—Phillips AN, Dunn D, Sabin S, Pozniak A, Matthias R, Geretti AM, Clarke J, Churchill D, Williams I, Hill T, Green H, Porter

K, Scullard G, Johnson M, Easterbrook P, Gilson R, Fisher M, Loveday C, Gazzard B, Pillay D. Risk of development of drug resistance in patients starting ART with 3 or more drugs in routine clinical practice. *Antiviral Therapy* 2004;9:S151. Abstract 135.

SUMMARY

This Rapid Report offered quick looks at 38 Resistance Workshop studies, including all oral presentations and a sprinkling of posters. But that tally represents a bare 23% of the meeting's reports. Files of many meeting posters will appear online at www.informedhorizons.com.

Suggesting headlines from a meeting rich in meaty results is a treacherous assignment accepted only by the highly hubristic or the irremediably ingenuous. The reader may decide which label applies:

- A once-daily regimen of ddI, tenofovir, and efavirenz failed fast in 6 of 14 treatment-naïve people, most of whom had high baseline loads and low baseline CD4s (abstract 156). Attendees suggested resistance mechanisms that may have torpedoed this threesome.
- Viral recombination rates proved surprisingly high in two separate studies by the National Cancer Institute resistance group in Frederick, Maryland (abstracts 44 and 45). One team suggested recombination may be as frequent as resistance itself.
- Working with an experimental protease inhibitor, Dutch researchers uncovered a resistance mechanism that does not rest on protease mutations (abstract 36). A piquant question is whether this mechanism explains other PI failures in mutation-free people.
- Two scientists who helped discover the mechanism of resistance to AZT suggested how novel compounds may exploit that mechanism to prevent or overcome AZT resistance (abstracts 26 and 31).

Research on new antiretrovirals confirmed the maxim that an antiretroviral without resistance is an antiretroviral without activity. Of the many agents reviewed, the PI tipranavir stands closest to regulatory review (abstract 147), with phase 3 results due out later this year. Studying women from Côte d'Ivoire who took single-dose nevirapine to ward off mother-to-child transmission, French researchers tied higher nevirapine blood levels to a greater risk of resistance (abstract 160). Meanwhile the HIVNET-012 team found loftier nevirapine resistance rates among Ugandan women with HIV-1 subtype D than among those with subtype A (abstract 50). Does resistance heighten the risk of death from HIV? In British Columbia, at least through 2001, the answer appears to be no (abstract 84), but in Italy yes (abstract 139).

EXAMINATION

The estimated time to complete this activity is 2 hours. In order to obtain credits, you should read the objectives and monograph, answer the 10-question multiple-choice post test, and complete the evaluation form; 70% or more of the answers must be correct to receive a certificate of completion.

- 1) **The prevalence of triple-class-resistant virus in the UK has:**
 - a) Remained stable from 1999 through 2002
 - b) Decreased from 1999 through 2002
 - c) Increased from 1999 through 2002
 - d) Remained stable or increased depending on the estimating method
- 2) **Decreased susceptibility to the investigational protease inhibitor Ro-033-4649 correlated with:**
 - a) A position 48 mutation shared with saquinavir
 - b) A unique mutation at protease position 71
 - c) Nucleotide changes in gag but not in protease
 - d) All of the above
- 3) **Two studies of the mechanism behind resistance to AZT suggest that:**
 - a) New agents may be developed to thwart this mechanism
 - b) Only the M184V mutation can ease resistance to AZT
 - c) Thymidine analog mutations are somewhat less likely with d4T
 - d) Resistance to AZT is essentially irreversible
- 4) **In a study of women who took single-dose nevirapine to prevent mother-to-child transmission of HIV, postpartum resistance patterns in the mother depended on:**
 - a) Age at delivery
 - b) HIV serostatus of the neonate
 - c) HIV-1 subtype
 - d) Gravidity
- 5) **Recent work indicates that, during failure of a lopinavir/ritonavir regimen, protease-resistant virus:**
 - a) Cannot emerge in previously naive patients because of a genetic bottleneck
 - b) Can emerge in previously naive patients, but does so rarely
 - c) Can emerge in previously naive patients, but only if they are not taking 3TC
 - d) Emerges in as many as 10% of patients
- 6) **A study of resistance and mortality in British Columbia found that:**
 - a) Most deaths occurred in people with highly resistant virus
 - b) Most deaths occurred in people without resistant virus or with a viral load below 500 copies/mL
 - c) Death was more likely among people who started treatment with monotherapy or dual therapy
 - d) Both a and c
 - e) Both b and c
- 7) **According to an analysis of treatment-naïve CATCH cohort members in Europe and Israel:**
 - a) The genetic barrier to resistance is similar in people with subtype B virus and in those with non-B subtypes
 - b) The genetic barrier to resistance is higher in people with subtype B virus than in those with non-B subtypes
 - c) The genetic barrier to resistance is lowest in recent immigrants from Africa
 - d) The genetic barrier to resistance is highest in Scandinavian countries
- 8) **In a study of tipranavir/ritonavir in people with abundant protease mutations:**
 - a) The 2-week virologic response was better with tipranavir/ritonavir than with other ritonavir-boosted protease inhibitors studied
 - b) People with 3 or 4 primary protease mutations at baseline responded better to tipranavir/ritonavir at 2 weeks than to other ritonavir-boosted PIs
 - c) Median viral loads began to rebound in all treatment arms after 2 weeks
 - d) All of the above

- 9) **In a study of 35 people enrolled in 4 different structured treatment interruption (STI) protocols:**
- a) Resistance emerged during STIs only among those stopping a nonnucleoside
 - b) Resistance proved most common during STIs in people stopping an unboosted protease inhibitor
 - c) Both archived mutations and new mutations emerged during STIs
 - d) Only archived mutations emerged during STIs
- 10) **A 2001-2003 study of antiretroviral resistance in Los Angeles, New Orleans, New York, and Seattle found that:**
- a) Prevalence of resistance decreased over the study period.
 - b) Prevalence of resistance to at least one antiretroviral class measured 49%.
 - c) Prevalence of resistance to at least one antiretroviral class measured 79%.
 - d) Resistance was most common in New York.

EVALUATION QUESTIONS

Objectives:

- Provide an update on resistance to new antiretroviral agents
- Review mechanisms of HIV drug resistance
- Present topics specific to HIV pathogenesis, fitness and resistance
- Expand the scope of the clinical implications of resistance
- Present new developments in epidemiology
- Introduce new resistance technologies and interpretations

11) **The program met the above listed objectives.**

- a) Strongly agree c) Neutral
- b) Agree d) Disagree

12) **Please list a concept that you have learned during this session and how you are going to apply it to your practice.**

13) **Overall, the quality of the activity was:**

- a) Excellent d) Fair
- b) Good e) Poor
- c) Average

14) **Based on your CME needs, please give suggestions for future program topics/formats.**

Please fax the completed examination to +1 858 534 7672

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