Is it Ever *TOO EARLY?*

Some say that with better ARVs available, it's time to treat all HIV-infected individuals immediately, and even argue that early treatment could help prime some people for a future functional cure. Others worry it may do more harm than good.

By Andreas von Bubnoff

"Time to hit HIV, early and hard"—that was the title of an editorial that appeared in 1995 in the *New England Journal of Medicine*. The author was David Ho, a researcher at the Aaron Diamond AIDS Research Center in New York City. Ho played a major role in developing combination antiretroviral therapy (cART, a.k.a. highly active ART, or HAART), which revolutionized AIDS care. "I remember it like it was yesterday," says Jens Lundgren, an infectious disease physician at the Copenhagen University Hospital. The essay, he recalls, appeared at a time when researchers were seeing the first cases of HIV-infected individuals improving from the therapy. "For the first time in my career, I saw a CD4[+ T] cell count increasing. The editorial was written in that atmosphere."

But it would take another 17 years before two expert panels—one convened by the US Department of Health and Human services (DHHS), the other called the International Antiviral Society (IAS)-USA—recommended for the first time that all HIV-infected individuals be treated, regardless of their CD4+ T-cell counts, as long as the patients were ready and willing to adhere to therapy. IAS-USA also recommended for the first time that anyone acutely infected with HIV should be offered ART, even in the absence of symptoms. In principle, the guidelines are relevant for all countries, says Melanie Thompson of the AIDS Research Consortium of Atlanta, who chaired the IAS-USA panel that wrote the 2012 recommendations. But their full implementation may not be possible in developing countries, due to limited resources (see *Research Briefs*, page 18).

One reason it took until this year for the expert panels' recommendations to catch up with Ho's editorial is that the risk of side effects, and of developing drug resistance to antiretrovirals (ARVs), was long viewed by many as too great to recommend early treatment for everyone, says Martin Markowitz, who is also at Aaron Diamond. But now the drugs have fewer side effects than ever and are so potent, easy to take, and diverse in their mechanisms that the risk of developing resistance to them has declined.

Further, Thompson says, a growing number of studies show that starting ART earlier has lasting benefits, enough to support treatment at all T-cell counts. For example, HPTN 052, a randomized trial in serodiscordant couples, showed that starting ART at CD4+ T-cell counts of 350-550 cells/µl is better than waiting until the count drops to 200-250 (*N. Engl. J. Med.* **365**, 493, 2011). Earlier treatment reduced HIV transmission by 96%, and the early starters had fewer AIDS-related illnesses. "[After] David Ho wrote that editorial, it took [17] years to show that treatment does prevent transmission," says Markowitz, referring to HPTN 052. The science, he says, had always suggested early treatment was the right way to go. "Now that the drugs have caught up with the science and the science has matured, it's a pretty simple argument."

What's more, research also suggests that treating people very early, in the first few weeks and months after infection, can prevent much of the initial destruction of the immune system and diminish the HIV reservoir. Some researchers even suggest that, if combined with therapeutic vaccines or drugs that target the reservoir, a very early start of ART could lead to a functional cure.

Still, some researchers are calling for more studies before treatment can be recommended for everyone. It's unclear, they say, whether lifelong ART is beneficial in the long run in people who start early,

as opposed to waiting to start until the CD4+ count has dropped to levels around 350. For now, the IAS-USA and the US DHHS guidelines are the only ones that recommend starting treatment regardless of CD4+ T-cell counts. Others, such as the European AIDS Clinical Society (EACS) guidelines, only recommend starting treatment below 350.

Therapy in the acute phase

A growing body of evidence suggests early treatment has its benefits. Markowitz, for example, recently reported that people who start ART about 50 days after infection have lower immune activation, suggesting that it might delay progress to disease (see *IAVI Report* online Special Feature article, *Cure Research: Marching on—but over uneven terrain*).

Others have found that early treatment reduces the size of the HIV reservoir. Perhaps the first evidence for this came from a 2005 study that showed less viral outgrowth in cultured, latently infected CD4+ T cells taken from patients who started ART within six months following infection than in such cells from patients who started ART during chronic infection (*J. Infect. Dis.* **191**, 1410, 2005).

A team led by Huldrych Günthard of the University Hospital Zurich, who was involved in that study, later showed that compared with patients who started ART during chronic infection, those who started therapy three to 15 weeks after infection had a roughly 10-fold smaller HIV DNA reservoir in their white blood cells (*PLoS One* **5**, e13310, 2010).

Steven Deeks and colleagues at the University of California, San Francisco, have made similar observations. They found a five-fold smaller reservoir in white blood cells taken from people who had started ART within six months after infection and were then treated for at least two years, compared with people who started ART later than two years after infection. What's more, Mathias Lichterfeld at Massachusetts General Hospital in Boston and his colleagues studied nine patients who started ART one to two months after infection, and then remained on treatment for 10-15 years. It was not possible to retrieve any replication-competent virus from the CD4+ T cells from many of these patients, even when using a large number of cells from their blood. Lichterfeld made similar observations in elite controllers.

Perhaps the largest study of early ART starters is being conducted by Jintanat Ananworanich and colleagues at the Thai Red Cross AIDS Research Center in Bangkok, the largest HIV testing clinic in Thailand. The researchers screened more than 50,000 patient samples to identify 77 patients between one and about four weeks after infection.

They found that the earlier treatment was started, the smaller the reservoir size in blood and colon six or 12 months later. In fact, after half a year of treatment, the 19 people who had started ART the earliest—one to two weeks after infection—had a reservoir size matching that of elite controllers. It was also about 10 times smaller than in people who started ART during chronic infection and were treated for five years. Intriguingly, it appears that the HIV DNA in these 19 patients had not integrated into the host's white blood cell genome, whereas patients who started ART just two weeks later than them did have integrated HIV DNA. "That shows that if you capture people really early, you may be able to block further integration," Ananworanich says.

Perhaps, Lichterfeld says, starting ART within the first week after infection can prevent the establishment of the reservoir. To test this idea, he says, investigators at the Ragon Institute are now setting up a study in Africa in which high-risk patients get tested for HIV RNA every week; those found to be positive will be put on ART immediately and followed to permit measurements of their viral reservoirs.

Path to a functional cure?

Some patients who start ART during acute infection seem to be able to control the virus after stopping therapy, suggesting that they may be functionally cured. At a meeting on cure research just before the International AIDS Conference earlier this year in Washington, D.C., Asier Sáez-Cirión, an assistant professor at the Institut Pasteur, reported that he and his colleagues have studied 14 such cases (see *IAVI Report* online Special Feature article, *Cure Research: Marching on—but over uneven terrain*).

Patients in this so-called VISCONTI cohort started therapy on average 39 days after infection. They were identified and recruited by researchers, who searched hospitals across France for patients who had been treated for at least a year before treatment interruption, and who subsequently controlled their viral load for at least a year. Although the search did not exclude people who started therapy during chronic infection, the researchers discovered that all 14 of the patients identified and recruited had started therapy during the acute phase of infection.

These were, in other words, post-treatment controllers and not elite controllers—those rare HIV-infected people who control the virus without any treatment at all. Indeed, Laurent Hocqueloux, an infectious disease doctor in Orléans,

France, who coordinates the studies of the VISCONTI cohort, says their HLA alleles differ from those of elite controllers. While elite controllers are more likely than most people to have HLA alleles such as B27 and B57 that somehow contribute to better control of the virus, the VISCONTI patients are less likely to have these alleles. They are, oddly enough, more likely to have the B35 allele, which is associated with poor control of viral load and faster progression to AIDS.

This, Hocqueloux says, could explain why 90% of the VISCONTI patients showed symptoms when they were acutely infected, a phenomenon that probably accounts for their early identification.

To get a better idea of how many people who start treatment early control viral load, the French researchers also searched thousands of cases in French hospital records for cases of post-treatment control. They found 74 patients who started treatment within six months after infection, were treated for at least a year, and then stopped treatment. Of those, about 15% were able to control infection for two years after treatment was stopped—a much higher percentage than the roughly 0.5% of elite controllers in the general population. To Hocqueloux, this suggests that early treatment is the major reason the VISCONTI patients can control viral load after interrupting treatment. He says he is now looking for markers that can predict which patients can become post-treatment controllers.

Not everyone is convinced. Günthard says that he too has seen a few patients who controlled viral load after starting treatment early and then interrupting it about a year and a half later, "but we didn't make a big story out of it." He doesn't think the effect is necessarily due to the early start of treatment; it's unclear, he points out, what would have happened if they hadn't been treated early or if they hadn't been treated at all. And even if post-treatment controllers differ from elite controllers, Günthard says, it's possible that they control viral load by unknown mechanisms that differ from elite control but are also unrelated to the early start of therapy. For example, he says, the effect could be due to differences in the viruses these people are infected with. He has found that viral differences can affect the viral load even more than differences in HLA alleles.

The exact mechanism of post-treatment control, if it's real, is indeed unclear. Sáez-Cirión recently reported that the VISCONTI volunteers have a smaller viral reservoir than people who start therapy later, during chronic infection. In some, the reservoir even seems to be shrinking. Charline Bacchus of the Pitié-Salpêtrière Hospital in Paris recently reported that one possible explanation for this is that the reservoir consists of an unusually small fraction of long-lived cells (see *IAVI Report* online Special Feature article, *Cure Research: Marching on—but over uneven terrain*).

Yet a small reservoir alone isn't sufficient for viral control, Hocqueloux says. Even people with extremely small reservoirs can't always control the virus without treatment. He and his colleagues are therefore looking for other explanations. One possibility, he says, is that early starters have healthier immune systems. Hocqueloux says he has some evidence that that could be the case.

Consistent with that, Ananworanich and colleagues found that if ART is started within the first few weeks after infection, just one year of treatment can reconstitute $CD4^+$ T cells to almost normal levels in the blood and the gut. This usually does not happen in people who start ART later, during chronic infection, she says.

Another effect of early ART is that it slows viral evolution by nearly halting HIV replication. Sarah Palmer and colleagues recently reported that individuals who started ART during acute infection have a less diverse viral population (see *Stalking HIV's Sleeper Cells*, *IAVI Report*, Mar.-Apr. 2012). This, Hocqueloux says, could make it easier for their immune systems to keep the virus in check. "Perhaps the preserved immune system and the genetic restriction of the virus together lead to control," he says. He plans to sequence viruses in the VISCONTI patients to see if theirs are less diverse as well.

Meanwhile, a team led by Christine Rouzioux from the University Paris Descartes has started enrolling patients in a trial called OPTIPRIM, designed to explore the induction of post-treatment control. All 90 trial participants are to start ART within 10 weeks after infection and are randomly assigned to one of two groups. One will get ART with a traditional three-drug ART regimen similar to the one used by the patients in the VISCONTI cohort. The other will get a more aggressive five-drug regimen that additionally includes the CCR5 inhibitor maraviroc and the integrase inhibitor raltegravir. The treatments will be stopped, with careful monitoring, after two years and the researchers will check whether the participants can control viral load. Any who fail to do so will restart treatment immediately.

Rouzioux and colleagues hope that the more effective five-drug regimen will induce control in a larger fraction of post-treatment controllers than the three-drug regimen, and result in a

larger reduction of the reservoir size. They will try to identify biomarkers that are associated with control. Rouzioux says they will also study patients who can't control the virus to see which ART regimen leads to a longer delay in viral rebound, and which better preserves immune responses and reduces immune activation.

Should the five-drug regimen create a significant proportion of post-treatment controllers, early ART followed by closely monitored treatment interruption to check for post-treatment control could even someday become standard clinical practice, Rouzioux says.

"No one in the clinic ever wants to stop therapy these days," says Deeks. "In general, once we start people on therapy, we never stop unless we have to." However, he adds, "if a mechanism for post-treatment control can be identified, and a biomarker that predicts outcome, then it is possible some people who are potentially destined to do well can stop drugs." In addition, he says, reducing the size of the reservoir in patients who start ART during acute infection might make a future cure more feasible for such patients.

To test this idea, Ananworanich and colleagues are already planning to combine early ART with other treatments that boost the immune system or target the reservoir to see if that might result in a functional cure. They will, she says, assign volunteers randomly either to an early three-drug or to a five-drug regimen and then interrupt treatment. Next, they will check if some can control viral load either without treatment, or after treatment with therapeutic HIV vaccines or drugs such as SAHA that activate the HIV reservoir. "We feel that these patients have the highest chance of achieving functional cure because they have such [a] low reservoir and their immune system is likely intact," Ananworanich says. "The early treatment is not the whole answer. It's just to get them to a stage that they have very little virus [and a] good immune system and then test another strategy."

A need for more evidence?

Most data that support starting ART during acute infection come from observational studies, not randomized trials. One reason, Thompson says, is that it's difficult to find enough acutely infected patients for large randomized trials and follow them long enough to see clinical effects such as diseases or mortality. Only a small percentage of all HIV-infected people are identified early because most acutely infected people just show nonspecific flu-like symptoms, or no symptoms at all.

Still, some of the data that supported the 2012 IAS-USA panel's recommendation to treat even acutely infected patients without any symptoms did come from randomized trials. For example, the 115-volunteer randomized Primo-SHM trial showed that, compared with untreated participants, six- or 15-month long ART started within about four weeks after infection lengthened by about 1-2.5 years the time HIV-infected individuals could stay without therapy before they reached a $CD4^+$ cell count of 350 or less (*PLoS Med.* **9**, e1001196, 2012). This means that treating people in the acute phase of HIV infection has an effect on the immune system, which gives them more time to disease progression, says Marlous Grijsen, a physician at the Academic Medical Center at the University of Amsterdam, who was involved in the study. She adds that important early treatment-related changes were $CD4^+$ cell gain and a lower viral setpoint.

Two other randomized trials also studied whether temporary ART that was started within the first six months after infection and stopped between three and 12 months later could delay when volunteers had to restart treatment. One of them, ACTG A5217 (also known as the setpoint study) showed this so clearly that it was stopped prematurely (*J. Infect. Dis.* **205**, 87, 2012). Preliminary results from the other trial, called SPARTAC, also point in the same direction.

Should the SPARTAC trial's final results show similar advantages of early treatment as the Primo and setpoint studies, HIV treatment guidelines should be changed to recommend immediate treatment for acutely infected patients, Grijsen says, provided the advantages and potential disadvantages such as side effects are discussed with each individual.

But others are not so sure. "It's unclear whether there is net benefit or net harm from starting therapy during the acute infection or in the early chronic stage of HIV as opposed to deferral of treatment until the $CD4^+$ cell counts have dropped to lower numbers," says Lundgren, who helped devise the EACS guidelines. "We do not know whether ART used during acute infection or in asymptomatic patients with high $CD4^+$ counts provides net benefit or net harm in terms of morbidity and mortality, compared with a strategy of deferring until the $CD4^+$ cell count has dropped to around 350." He says the EACS guidelines, which currently do not recommend treatment above a $CD4^+$ cell count of 350 unless there are other health issues, are written this way in part because there is no randomized clinical trial that shows that non-fatal disease over-

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all (for example, kidney disease), is lower if you treat early than if you treat a little later. Even though today's ARV drugs have far fewer side effects than they used to, he says, "we are running the real risk that there is net harm from using [ART] early in the course of HIV disease."

Even the current drugs have side effects such as bone density loss, and cardiovascular, liver, and kidney damage, adds Günthard. "If this would have the same effect as drinking milk, then there would be no question. But it's not milk." Still, he adds, most observational data do suggest that early treatment is beneficial with respect to non-AIDS defining illnesses such as cancer and cardiovascular disease. So despite the uncertainties, Günthard says he strongly favors early treatment.

But Lundgren and others have initiated a randomized trial called START that, they say, will show if there are any clinical net health benefits—relative to drug side effects and toxicity—from taking the drugs earlier. The 4,000-person trial will, for the first time, examine whether the net health benefits are different if patients start ART above 500 $CD4^+$ T cells or defer treatment until $CD4^+$ counts have dropped to levels below 350. For about five years after patients are enrolled, researchers will monitor patients for AIDS-related and for serious non-AIDS-related events such as heart attacks, stroke, kidney disease, or liver disease. Some of these are known to be side effects of drugs and others are known to be HIV-related.

Not everyone wants to wait that long for answers. Julio Montaner, who runs a program that tests and offers immediate treatment to infected people in British Columbia, says he doesn't need additional data. "[In] every clinical trial, always the higher $CD4^+$ group wins," he says. "How much more evidence do you need before you recommend treatment to all?"

Still, testing is key. Even if very early treatment of acute infection turns out to have benefits for HIV-infected individuals, such as bringing them closer to a cure, these can only be realized if infection is detected early enough. Currently, however, only a small proportion of people are identified during acute infection, says Lichterfeld. "If you were to treat everybody in acute infection, it wouldn't make a huge difference, because the proportion of patients that would be eligible would be very small. So it would not be a major intervention to cure HIV." ■