HARRY W. KESTLER, PhD
Dr. Kestler is an assistant staff member in the Department of Molecular Biology in the Research Institute of the Cleveland Clinic, and the principal investigator for the LIVE project (*Live Immunodeficiency virus Vaccine Evaluation*).

BIMAL K. CHAKRABARTI, PhD
Dr. Chakrabarti is a research fellow in the Department of Molecular Biology and a LIVE project investigator.

A live-virus "suicide" vaccine for human immunodeficiency virus

ABSTRACT: A vaccine that uses a live, attenuated human immunodeficiency virus (HIV) may offer the best hope of a vaccine against acquired immunodeficiency syndrome (AIDS). A recent improvement should increase the safety of the live-virus approach: a "suicide gene" inserted into the viral RNA, which causes infected cells to die when treated with ganciclovir. We envision using this strategy not only to prevent AIDS, but also to treat it.

Live vaccines for AIDS are becoming more acceptable in principle, because other vaccines have failed

Vaccines that use live, attenuated viruses have been important weapons against viral diseases ever since the 1700s, when Jenner[1] used a live vaccine to prevent smallpox. Forty years ago, Sabin[2] discovered that some healthy persons harbored a strain of polio virus that appeared to confer protection against poliomyelitis. The discovery led to a vaccine consisting of weakened, or attenuated, polio virus, which has dramatically reduced the incidence of poliomyelitis. Since then, many successful attenuated-virus vaccines have been created (TABLE 1).

Live attenuated vaccines induce stronger immune responses than do vaccines consisting of viral fragments or killed viruses, because in the body they act like the natural, virulent strains they protect against. In particular, the viruses in live vaccines can reproduce and mutate, presenting the immune system with a variety of antigens and generating a broad immune response.

Such an immune response will be necessary to stop an infection with the human immunodeficiency virus (HIV), which mutates rapidly to evade the immune response. Evidence of this rapid mutation comes from work by Burns and Desrosiers,[3] who injected rhesus monkeys with simian immunodeficiency virus (SIV), a lentivirus similar to HIV. Even though the virus was molecularly cloned and thus uniform when injected, the researchers recovered many variants of the clone from the infected animals. In essence, the lentiviruses such as HIV present the immune system with a "moving target" by changing their surface antigens rapidly.

TABLE 1

DISEASES CONTROLLED BY LIVE ATTENUATED VIRAL VACCINES

- Smallpox
- Polio
- Measles
- Mumps
- Rubella
- Dengue
- Chicken pox
- Influenza
- Parainfluenza type 3
- Respiratory syncytial virus infection (in development)
- Acquired immunodeficiency syndrome (proposed)

TABLE 2

MILESTONES IN DEVELOPING A LIVE-VIRUS AIDS VACCINE

Year	Development
1989	First pathogenic AIDS virus clone isolated[8]
1991	Simian immunodeficiency virus (SIV) from which the *nef* gene has been deleted is created in the laboratory; animals infected with *nef*-attenuated virus do not develop AIDS[9]
1993	Animals infected with SIV lacking *nef* survive challenge with pathogenic SIV[10]
1995	Long-term AIDS survivors are shown to harbor HIV lacking *nef*; all are healthy, and at least one had multiple exposure to HIV[14,15]
1996	A suicide gene is added to HIV and SIV lacking *nef* to add safety and to enable the development of a gene therapy for AIDS[24]

WOULD A LIVE-VIRUS HIV VACCINE BE SAFE?

We believe we can use molecular biology to make a safe, live-virus vaccine against HIV

Although live-virus vaccines are more effective than other types, they also pose the most risk of causing the very diseases they are designed to prevent. A few cases of poliomyelitis occur each year as a result of polio vaccination, but the benefit to society outweighs the risk. However, HIV is different: so deadly is HIV infection that the thought of causing a single iatrogenic case is horrific.

Nevertheless, live vaccines for AIDS are becoming more acceptable in principle, if only because other vaccines have failed. Vaccines that used whole inactivated or killed virus did not prevent infection in animal models,[4] although inoculated animals progressed to AIDS slower than did controls.[5] Additionally, whole inactivated vaccines have safety problems associated with the methods of inactivation. Subunit vaccines presented as purified proteins or as proteins expressed on an alternative virus backbone have also not induced protective responses.[6]

We believe we can use the techniques of molecular biology to make a safe, live-virus vaccine against HIV.

HOW TO BUILD A LIVE-VIRUS VACCINE

Up to now, most live attenuated vaccines were created by propagating the virus in vitro in well-characterized cells. In contrast, live vaccines for AIDS use modern recombinant DNA technology.

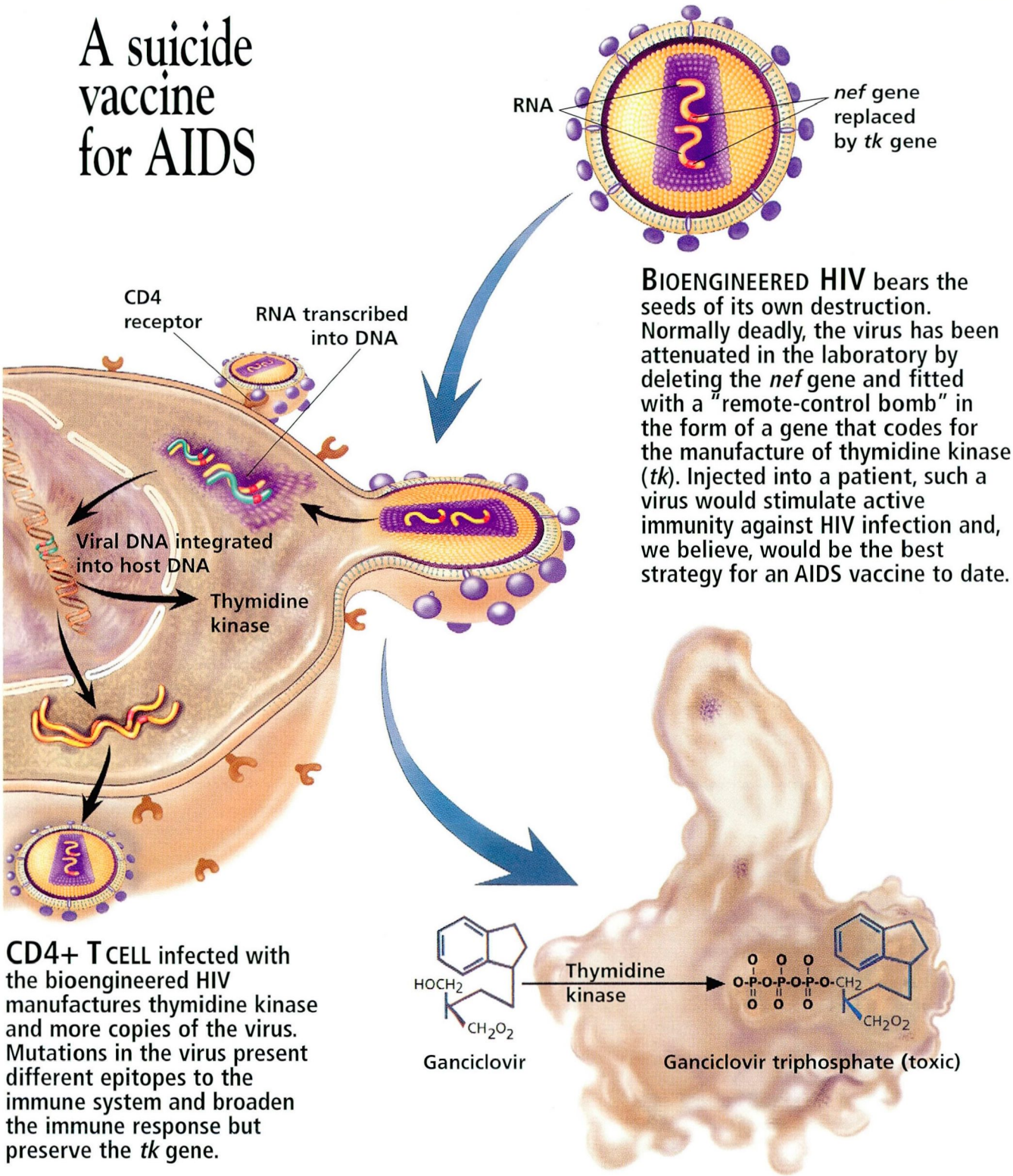
Step 1: Clone viral DNA from infected T cells

Primate lentiviruses such as HIV are retroviruses and thus carry their genetic information in the form of RNA rather than DNA. Genetic manipulation of RNA is technically very difficult. However, once inside a host, a retrovirus transcribes its RNA onto DNA, which the host integrates into its genome.

This DNA copy can be isolated and manipulated. In 1989, DNA was isolated from T cells of rhesus monkeys infected with SIV, and the viral DNA was cloned.[7] This molecular cloned virus—SIVmac239—not only reproduced when transfected to uninfected cells, it induced AIDS in rhesus monkeys.[8] This clearly demonstrated that genetic information contained in AIDS viruses can induce AIDS (TABLE 2). (Despite this clear demonstra-

FIGURE

A suicide vaccine for AIDS



BIOENGINEERED HIV bears the seeds of its own destruction. Normally deadly, the virus has been attenuated in the laboratory by deleting the *nef* gene and fitted with a "remote-control bomb" in the form of a gene that codes for the manufacture of thymidine kinase (*tk*). Injected into a patient, such a virus would stimulate active immunity against HIV infection and, we believe, would be the best strategy for an AIDS vaccine to date.

CD4+ T CELL infected with the bioengineered HIV manufactures thymidine kinase and more copies of the virus. Mutations in the virus present different epitopes to the immune system and broaden the immune response but preserve the *tk* gene.

GANCICLOVIR OR ACYCLOVIR, given at a specified time after immunization, detonates the "bomb" and clears the bioengineered HIV from the body. Thymidine kinase in infected cells metabolizes the drug into a toxic product, killing the cell. In the "bystander effect," nearby cells are killed as well.

tion, some people continue to question whether AIDS is caused by a virus.)

Step 2: Delete the *nef* gene to attenuate the HIV

In further work, we deleted a single gene (*nef*—*ne*gative *f*actor) from SIV. This gene was incorrectly named: it does not appear to be a negative factor but a positive factor for viruses containing it. The molecular mechanism by which *nef* acts is poorly understood, but it does appear to influence the ability of the virus to spread in infected individuals and it facilities the replication of the virus in poorly dividing or nondividing T cells.

Injected into adult rhesus monkeys, the altered virus established a lifelong infection just as a natural strain would, with one critical difference: viral loads remained at least 2 log lower. None of the monkeys contracted AIDS, unlike control monkeys infected with SIV having a functional *nef* gene. Further, the animals infected with the altered virus all developed a robust immune response to SIV,[9] and were protected from superinfection with disease-inducing strains of SIV.[10] These results led to the concept of a live attenuated vaccine for AIDS.

Some long-term asymptomatic AIDS survivors harbor HIV without the *nef* gene

SIV from which the *nef* gene has been deleted has an attenuated phenotype that is stable, even though *nef* gives SIV a tremendous selective advantage for replication.[11] Once this gene is deleted, its information is gone, and the virus cannot grow another one.

There is some controversy about the pathogenicity of *nef*-attenuated SIV in neonates, who may lack the cellular immune responses needed to control the initial bout of viremia. Ruprecht and colleagues[12] infected neonate rhesus monkeys and showed that viruses from which *nef* had been deleted retained the ability to cause disease. However, Wyand et al[13] have found minimal pathogenesis in neonates infected with the attenuated strain. Clearly, persons lacking a complete set of immune responses (ie, neonates, immunosuppressed transplant recipients, AIDS patients) are not likely to benefit from a live attenuated vaccine. However, the suicide vaccine could be used to control viral loads in persons who inappropriately acquire the vaccine strain, such as neonates infected from a sibling or women who discover they are pregnant after being vaccinated.

The *nef* gene probably serves a similar function in HIV as it does in SIV; thus, the results in the rhesus monkey model are applicable to humans.

Recent observations support the validity of this animal model. Two groups have discovered that some long-term asymptomatic AIDS survivors harbor HIV without the *nef* gene, suggesting that *nef* is also a pathogenic determinant in HIV.[14,15] Perhaps, as was the case for polio,[2] there are naturally occurring attenuated mutations of HIV that confer protection to persons who harbor them.

The next generation of attenuated vaccines: add a suicide gene

Temin[16] and Desrosiers[17] independently proposed that deleting other viral genes, in addition to *nef*, would increase the safety of an HIV vaccine. However, additional deletions might cripple the vaccine strain to the point where it no longer induces a robust antiviral immune response.

We chose a different approach that does not further attenuate the vaccine strain but potentially enables additional control of the virus. We placed a conditional lethal genetic element, or "suicide gene," into *nef*-deleted AIDS viruses.[18] The gene we chose was the thymidine kinase (*tk*) gene from the herpes simplex virus (FIGURE).[19]

Herpes viruses can be controlled with acyclic nucleoside analogues, such as ganciclovir or acyclovir.[20,21] The *tk* gene causes infected cells to manufacture thymidine kinase, which metabolizes these drugs into a toxic product, killing the cell.

The *tk* gene can also function when inserted into other types of viruses. This conditional gene, delivered into the target virus by retroviral vectors, has been used successfully as a gene therapy for cancer and for cell proliferation due to vascular damage.[22,23]

Ganciclovir controls genetically altered HIV and SIV

In recent work, we replaced the HIV *nef* gene

with a herpes *tk* gene and found that such an altered strain of HIV expressed the *tk* gene well without any further modifications.[24] Another group has obtained similar results.[25]

In some ways, HIV is easier to alter in this way than is SIV. Since the SIV *nef* gene overlaps with the envelope gene, we could not replace it completely. (Such an overlap does not occur in HIV.) Therefore, we deleted the portion of the *nef* gene that did not overlap, made deletions on the remaining portion of *nef* to inactivate it, and made further mutations in the virus to enhance the expression of *tk*.

We tested the SIV and HIV viruses containing the *tk* suicide gene for their ability to confer sensitivity to ganciclovir in a variety of cell lines and peripheral blood mononuclear cells (Salkowitz, Chakrabarti, and Kestler, unpublished data).

The suicide gene is stable

The stability of the suicide gene is of considerable importance to the use of the live attenuated vaccine to prevent AIDS. If a live HIV vaccine with a *tk* gene rapidly mutated into strains resistant to treatment with ganciclovir, it would doom the approach. Such mutations are likely for two reasons.

Since treating HIV with antiretroviral compounds that target reverse transcriptase (which is necessary for completion of the virus life cycle) produces resistant mutants, such resistant mutants should be expected in a foreign gene such at *tk*.

In addition, mutations that inactivate *tk* in HIV are likely, since ganciclovir-resistant mutants arise in herpes virus. This occurs even though thymidine kinase provides a survival benefit to herpes virus,[26] and the herpes virus makes fewer replication errors than HIV.[27]

Despite our low expectations for the stability of the *tk* gene in HIV, we found that it remained functional, even after prolonged culturing, except in sublethal levels of ganciclovir.[24]

Perhaps the *tk* gene helps HIV survive, much as it helps the herpes virus (in the absence of ganciclovir). The *tk* gene may help HIV replicate faster by increasing the amount of nucleotides—the precursors of DNA—through thymidine and guanidine phosphorylation, as it does for herpes simplex virus in latent infections.[28] Wain-Hobson and colleagues[29] showed that manipulation of nucleotide pools can enhance the replication kinetics of HIV. Cellular thymidine kinase levels have been shown to be depressed in peripheral blood cells from HIV-seropositive persons.[30] This depletion may render HIV less susceptible to nucleoside analogues such as zidovudine. The cause of this decrease is unknown, but perhaps the addition of a herpes virus *tk* gene can complement the defect.

A TREATMENT FOR AIDS?

Although a live attenuated HIV suicide virus might be useful to prevent AIDS, such viruses might be used as a treatment for AIDS as well.

One strategy would not require any further modification of the suicide virus. When a herpes simplex virus *tk* gene is introduced into a cell, ganciclovir treatment kills not only the transduced cell, but also neighboring cells in a "bystander effect."[22,23] How this happens is unknown; one theory holds that phosphorylated ganciclovir diffuses out of the transduced cell and is taken up by neighboring cells. How close the neighboring cells must be to the transduced cell to be killed is also unclear.

All AIDS viruses target lymphoid tissue soon after infection.[31] We reasoned the suicide vaccine would superinfect cells in the lymph nodes that are already infected with pathogenic HIV. Then ganciclovir would kill them and neighboring cells as well, which are also likely to harbor pathogenic HIV. The hope would be that this action may tip the balance to control the extent of the infection.

The suicide vaccine might be used as a gene therapy for AIDS

In designing vaccine virus strains we removed a gene that produces a barrier to superinfection,[32] so that cells infected with the suicide vaccine virus also can be superinfected by pathogenic HIV. This would allow cells infected with HIV-*tk* or SIV-*tk* to have the potential to absorb free virus and kill it. This therapy, superinfection with an attenuated suicide vaccine followed by ganciclovir treatment, could be repeated on multiple occasions, reducing the HIV viral load.

Additional improvements for the therapeutic use of attenuated AIDS viruses containing a suicide gene are anticipated. Such improvements would include the use of genetic selection strategies to obtain virus that target preinfected cells, enabling the specific delivery of HIV-*tk* to HIV-infected cells. ■

ACKNOWLEDGMENTS: The authors thank Robert J. Butkowski, Jamie Magid, and Janelle R. Salkowitz for helpful comments and suggestions and to Julie Kestler and Dorthy Herzberg for editing this paper. This work was sponsored by research grant #001988 from the American Foundation for AIDS, student intern award #62-96 from the Pediatric AIDS Foundation, research grant 50668 from the Pediatric AIDS Foundation, and grant IRG 63653 from the American Cancer Society.

REFERENCES

1. Jenner E. An inquiry into the causes and effects of the variolae vaccina, a disease discovered in some of the western counties of England, particularly Gloucestershire, and known by the name of cow-pox. 1798. Reprinted by Cassell and Co. LTD, 1896. Available in pamphlet vol. 4232, Army Medical Library, Washington, DC.
2. Sabin AB. Present status of attenuated live-virus poliomyelitis vaccine. JAMA 1956; 162:1589–1596.
3. Burns DP, Desrosiers, RC. Selection of genetic variants of simian immunodeficiency virus in persistently infected rhesus monkeys. J Virol 1991; 65:1843–1854.
4. Putkonen P, Nilsson C, Hild K, et al. Whole inactivated SIV vaccine grown on human cells fails to protect against homologous SIV grown on simian cells. J Med Primatol 1993; 22(2-3):100–103.
5. Hirsch VM, Goldstein S, Hynes NA, et al. Prolonged clinical latency and survival of macaques given a whole inactivated simian immunodeficiency virus vaccine. J Infect Dis 1994; 170(1):51–59.
6. Graham BS, Wright PF. Candidate AIDS vaccines. N Engl J Med 1995; 333(20):1331–1339.
7. Kestler HW, Li Y, Naidu YM, et al. Comparison of simian immunodeficiency virus isolates. Nature 1988; 331:619–621.
8. Kestler H, Kodama T, Ringler D, et al. Induction of AIDS in rhesus monkeys by molecularly cloned simian immunodeficiency virus. Science 1990; 248:1109–1112.
9. Kestler HW, Ringler DJ, Mori K, et al. Importance of the *nef* gene for maintenance of high virus loads and for the development of AIDS. Cell 1991; 191:651–662.
10. Daniel MD, Kirschhoff F, Czajak SC, Sehgal PK, Desrosiers RC. Protective effects of a live attenuated SIV vaccine with a deletion in the *nef*. Science 1992; 258:1938–1941.
11. Kirschhoff F, Kestler HW, Desrosiers RC. Upstream sequences in SIV are selectively deleted *in vivo* in the absence of *nef* function. J Virol 1994; 68:2031–2037.
12. Baba TW, Jeong YS, Pennick D, Bronson R, Greene MF, Ruprecht RM. Pathogenicity of live attenuated SIV after mucosal infection of neonatal macaques. Science 1995; 267:1820–1825.
13. Wyand MS, Manson KH, Lackner AA, Desrosiers RC. Resistance of neonatal monkeys to live attenuated vaccine strains of simian immunodeficiency virus. Nature Medicine 19973; 3:32–36.
14. Deacon NJ, Tsykin A, Solomon A, et al. Genomic structure of an attenuated quasi species of HIV-1 from a blood transfusion donor and recipients. Science 1995; 270:988–991.
15. Kirschhoff F, Greenough TC, Brettler DB, Sullivan JL, Desrosiers RC. Brief report: absence of intact *nef* sequences in a long-term survivor with nonprogressive HIV-1 infection. N Engl J Med 1995; 332(4):228–232.
16. Temin HM. A proposal for a new approach to a preventive vaccine against human immunodeficiency virus type 1. Proc Natl Acad Sci U S A 1993; 90:4419–4420.
17. Desrosiers RC. HIV with multiple gene deletions as a live attenuated vaccine for AIDS. AIDS Res Hum Retroviruses 1992; 8:1457.
18. Kestler HW, Jeang K-T. The safety of a live attenuated vaccine for AIDS. Science 1995; 270:1219.
19. Sullivan V, Talarico CL, Stanat SC, Davis M, Coen DM, Biron KK. A protein kinase homologue controls phosphorylation of ganciclovir in human cytomegalovirus-infected cells. Nature 1992; 358(6382):162–164.
20. Oxford JS. Inhibition of herpes virus by a new compound—acyclic guanosine. J Antimicrob Chemother 1979; 5(4):333–334.
21. Teare EL, Clements MR. Acyclovir for suspected systemic herpes infections. Lancet 1980; 1(8158):42.
22. Culver KW, Ram Z, Wallbridge S, Ishii H, Oldfield EH, Blaese RM. In vivo gene transfer with retroviral vector-producer cells for treatment of experimental brain tumors. Science 1992; 256(5063):1550–1552.
23. Ohno T, Gordon D, San H, et al. Gene therapy for vascular smooth muscle cell proliferation after arterial injury. Science 1994; 265(5173):781–784.
24. Chakrabarti BK, Maitra RK, Ma X-Z, Kestler HW. A candidate live inactivatable attenuated vaccine for AIDS. Proc Natl Acad Sci U S A 1996; 93:9810–9815.
25. Smith SM, Markham RB, Jeang K-T. Conditional reduction of HIV-1 replication by a gain of HSV-1 thymidine kinase. Proc Natl Acad Sci U S A 1996; 93:7955–7960.
26. Baldanti F, Silini E, Sarasini A, et al. A three-nucleotide deletion in the UL97 open reading frame is responsible for the ganciclovir resistance of a human cytomegalovirus clinical isolate. J Virol 1995; 69(2):796–800.
27. Ji JP, Loeb LA. Fidelity of HIV-1 reverse transcriptase copying RNA in vitro. Biochemistry 1992; 31:954–958.
28. Tenser RB. Role of herpes simplex virus thymidine kinase expression in viral pathogenesis and latency. Intervirology 1991; 32:76–92.
29. Meyerhans A, Vartanian JP, Hultgren C, et al. Restriction and enhancement of human immunodeficiency virus type 1 replication by modulation of intracellular deoxynucleoside triphosphate pools. J Virol 1994; 68:535–540.
30. Jacobsson B, Britton S, He Q, Karlsson A, Eriksson S. Decreased thymidine kinase levels in peripheral blood cells from HIV-seropositive individuals: implications for zidovudine metabolism. AIDS Res Hum Retroviruses 1995; 11:805–811.
31. Pantaleo JG, Graziosi C, Butini L, et al. Lymphoid organs function as major reservoirs for human immunodeficiency virus. Proc Natl Acad Sci U S A 1991; 88:9838–9842.
32. Salkowitz JR, Chakrabarti BK, Yen-Lieberman B, Starkey C, Bendele T, Kestler HW. The *nef* gene of SIVmac239 is necessary for efficient growth in H9 cells. J Biomed Sci 1996; 3:422–434.

ADDRESS REPRINT REQUESTS to Harry W. Kestler, PhD, Department of Molecular Biology, Research Institute, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195.