tion—is in fact seen during evolutionary adaptation. Whether in yeast adapting to a new environment (*11*) or in cancer cells escaping growth-control mechanisms (*12*), rare "driver" mutations arise in a background of "passenger" mutations that have a negligible impact on fitness. A common strategy to identify driver mutations is to find recurrent patterns across independent laboratory evolution experiments or cancer cell lines, such as mutations falling in the same genes. But to identify functional phosphosites, alternative approaches must be envisioned because evolution cannot be replayed multiple times. The work of Studer *et al.* opens new avenues in this respect. They reveal that lineage-specific preferences in phosphosite context have arisen across the fungal evolutionary tree. For example, proteins in the baker's yeast lineage show a depletion in proline-based phosphosite context and an increase in negatively charged context. They also found that specific classes of proteins acquired phosphosites in a coordinated fashion during specific time periods. These analyses suggest that global properties of phosphoproteomes are selected and therefore could be used to predict functional phosphorylation events. Equally important to predicting function will be our ability to filter out noise, which will require a more systematic consideration of protein abundance (*10*) and phosphorylation stoichiometry (*13*). More generally, the large data set of ancient sites identified in this work will make it possible to contrast structural and cellular properties of ancient and young sites (such as structural environment, presence of specific motifs, substrate interactions, expression, and localization) to discover new mechanisms and circuits involved in functional versus noisy phosphorylation.

Evolutionary cell biology is still in its early days (*1*). Thus, comparative proteomics efforts will be increasingly important to complement the postgenomic revolution, elucidate molecular differences between cell machineries across species, and fuel our understanding of life and its history. ■

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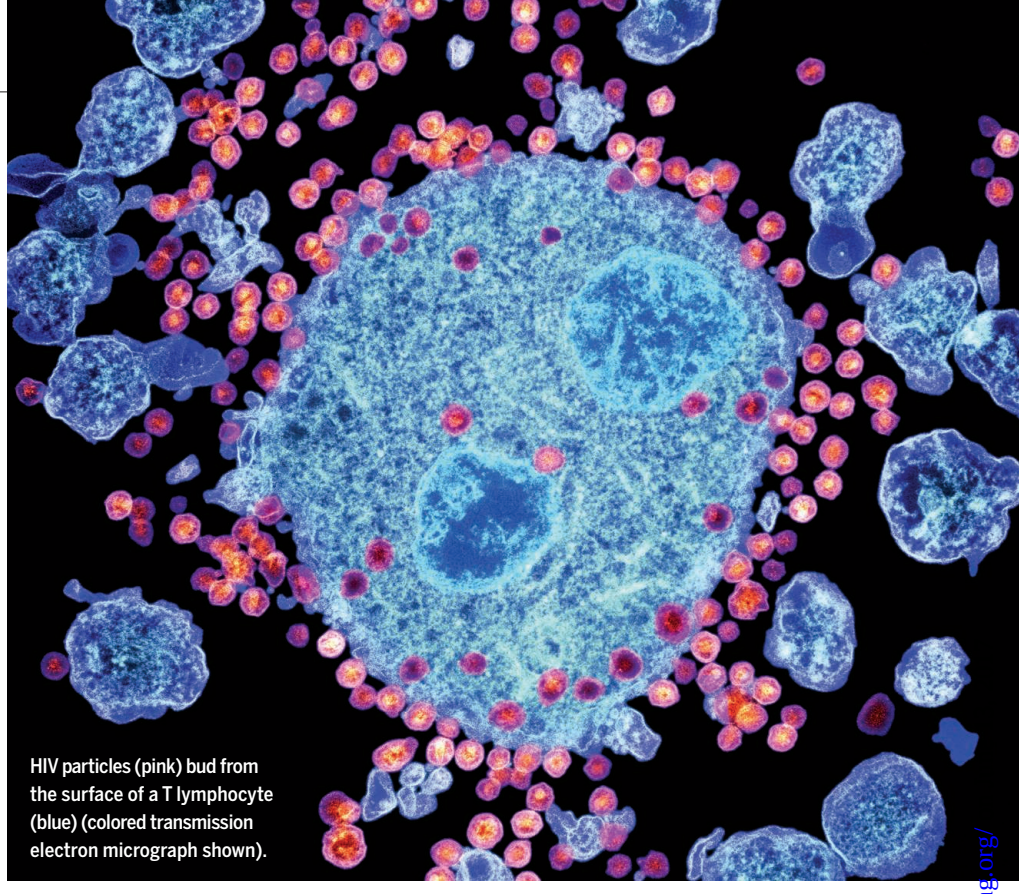

HIV particles (pink) bud from the surface of a T lymphocyte (blue) (colored transmission electron micrograph shown).

PHOTO: NIBSC/SCIENCE SOURCE

HIV

# *Shock and kill with caution*

## Strategies to silence latent HIV infection should be explored

*By* **Robert C. Gallo**

Antiretroviral therapy has made lifelong suppression of human immunodeficiency virus (HIV) replication a possibility for some patients. But with the 2015 estimate of 36.7 million people infected worldwide, there is a great need to explore other ways to address this epidemic—from preventing new infections by treating uninfected high-risk individuals, to developing a vaccine, to targeting latent HIV that hides in immune cells and persists in patients. The idea of clearing latent infection has prompted strategies aimed at an HIV-1 cure. Although this approach should continue to be tested, other approaches, including those that seek to permanently suppress the latent virus, should also be explored. Different strategies may target different viral reservoirs, and may turn out to be complementary.

The existence of T cells harboring latent HIV-1 provirus was first described in 1986 (*1*), as was the demonstration that activation of those T cells "reawakened" HIV-1 expression (*2*). The importance of these latently infected cells, however, came to the forefront following the work of several clinical investigators (*3*) who showed that these cells persist long after anti-HIV therapy and virus suppression, and periodically release HIV-1, presumably in response to a milieu favorable to T cell activation. The idea then arose that HIV-1 infection would be curable if latent virus could be deliberately reactivated. This would lead to T cell death, either directly from HIV-1 cytopathic effects or by cytotoxic T lymphocytes (CTLs). Concurrent anti-HIV therapy would block new rounds of infection. This idea has been called "shock and kill" or "kick and kill," and has spawned numerous studies (*4*), clinical trials, and discussions at meetings. Part of this impetus was provided by an enduring focus on one patient, the so-called "Berlin patient" (*5*), who—through a series of fortunate events—is the only known example of complete HIV-1 cure. This individual was HIV-1–positive, but also had leukemia, which allowed physicians to treat him with total body irradiation. Fortunately, he survived this aggressive treatment, and his leukemia.

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Another fortunate circumstance was the availability of bone marrow cells from an HIV-1–negative donor who carried a rare homozygous mutation in the HIV-1 co-receptor CCR5 (CCR5Δ32), which renders cells resistant to HIV-1 infection (*6*). With the ablation of his own bone marrow cells and the acceptance by his weakened immune system of T cells lacking functional CCR5, he became free of HIV-1 and presumably unable to be infected. Although no new principles came from this study, it did provide a proof of concept for a "cure." The results are indeed germane to developing approaches, such as gene therapy, that destroy or remove CCR5 from infected persons (*7*). However, the study's relevance to almost all other HIV-1–infected patients, as well as its connection to currently envisioned "shock and kill" approaches, is not apparent.

Clinical testing of the "shock and kill" approach has begun only recently, and therefore merits further evaluation (*8*). However, this strategy presents a few issues of concern that should not be overlooked. It is assumed that all latently infected cells will die following viral reactivation and/or by CTL attack, but there is no evidence for this; rather, these cells do not die. This may be because only weak latency-reversing agents (LRAs) have been tested clinically. Therefore, it is possible that more effective agents (alone or in combination) might do a better job at reawakening the virus and killing the infected cell, which should be tested. Also, there is no assurance that combination antiretroviral therapy (cART) will completely block the released virus from infecting new cells, especially in the brain or lymphoid tissues where the antiretroviral drugs may not reach fully suppressive concentrations. The effectiveness of these drugs in anatomical sites is still being debated. Treatment intensification does not seem to reduce the size of the viral reservoir, and there appears to be no genetic evolution of the virus in patients under suppressive cART therapy, both of which suggest that current regimens completely block virus spread. However, some have suggested that poor drug penetrance in certain anatomical sites plays against drug-resistant strains. Because this issue is still unresolved, we should use caution and consider that current regimens may not achieve suppressive concentrations (in at least some anatomical sites), and thus may be unable to block virus spread in the context of "shock and kill" strategies.

Another issue of concern is that some LRAs may activate uninfected T cells, rendering them new targets for infection. This could possibly be the case with Toll-like receptor agonists that are being evaluated as LRAs. More recently, the idea has emerged to combine certain LRAs with therapeutic vaccines, as a new version of the "shock and kill" approach. This is of course attractive, but it remains to be established whether a therapeutic vaccine can be devised to raise effective CTL responses in immunocompromised individuals. Indeed, in one case, a vaccine could raise protective CTL responses prior to infection (*9*), but its efficacy in infected patients is questionable.

Latent viruses other than HIV-1 may also be reactivated and spread by LRAs, thereby giving rise to serious side effects later on. Such viruses include Epstein-Barr virus, cytomegalovirus, human T cell lymphotrophic virus, hepatitis C virus, human herpes virus, human herpes virus 8 (Kaposi sarcoma herpes virus), and human endogenous retroviruses. Moreover, the use of agents that target host factors (all LRAs tested so far fall into this category) will also modulate host gene expression (including inheritable epigenetic marks), which may cause unwanted side effects. Indeed, any approach involving T cell activation could be toxic beyond just the potential of activating other disease-causing viruses and making cells more sensitive to HIV-1 infection. We should not forget that although cART does not represent a cure, it allows HIV-1–infected individuals a full life, especially with simpler drug regimens.

***"'Shock and kill' and 'soothe and snooze' may turn out to be two non–mutually exclusive approaches to a functional cure for HIV-1 infection."***

There are HIV-1–infected cell types other than $CD4^+$ T cells that are not given consideration in the "shock and kill" approach. For example, macrophages are infected by HIV-1 (*10*) and generally do not die from infection. Brain cells are also an HIV-1 sanctuary (*11*), mainly in microglial cells. A recent study has shown that viral replication persists in lymphoid tissues of infected individuals despite suppressed viremia (*12*). Although not directly addressed, myeloid cells could be the source of this persistent viral replication. Indeed, macrophages are a source of continual low-level production of HIV-1 (*13*). Whether or not myeloid cells represent a long-lived viral reservoir remains to be conclusively addressed, but until then, their role should not be ignored or discounted. In short, there is more to HIV-1 persistence in the context of suppressive cART than $CD4^+$ T cell reservoirs.

Agents such as histone deacetylase inhibitors show limited efficacy in reactivating HIV-1 because they target only one molecular mechanism that maintains viral latency. Many other blocks are still in place, including the lack of key transcription factors, the presence of other epigenetic blocks, and a cellular environment that is quiescent and unable to support viral replication.

Another approach to address latent HIV infection is to force the latent virus to remain quiescent by stronger and more durable virus-suppressing agents. So far, the only example of this "soothe and snooze" strategy is the use of the HIV-1 Tat inhibitor, didehydro-cortistatin A (*14*). This drug appears to be highly specific for Tat, without off-target effects. However, other latency-promoting agents, particularly those acting in an HIV-1–specific manner, should be explored. Other goals in developing this strategy include drugs targeting cellular factors that play a role in viral replication, and those that reach anatomical sanctuaries more efficiently. Durable HIV suppression could be concurrently supported by gene therapy strategies that target the HIV-1 provirus and/or CCR5 with gene-editing tools. In line with longer-term virus suppression, Byrareddy *et al.* (*15*) report that combining ART with antibodies that block T cells sustained low viremia in simian immunodeficiency virus (SIV)–infected monkeys.

Despite the success of cART, there are still very high-priority areas that must be supported, including HIV-1 testing, promoting preexposure prophylaxis, and developing a vaccine (*16*). Therapeutic strategies aimed at an HIV-1 cure through viral reactivation should continue to be tested, but other strategies such as viral suppression should also be explored. Indeed, funding agencies are starting to support research aimed at permanently silencing the latent provirus. "Shock and kill" and "soothe and snooze" may turn out to be two non–mutually exclusive approaches to a functional cure for HIV-1 infection. ■



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