tackling public-health issues in economically disadvantaged areas where malnutrition-associated immunodeficiency and infectious diseases remain widespread.

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HIV

Shock and kill

Antiretroviral therapies block HIV replication but they do not eliminate inactive viruses within cells. A clinical trial shows that a drug can revive HIV in patients as a potential first step towards a cure. See Letter p.482

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HIV therapeutics is about to enter a new phase. Over the past 25 years, the focus has been almost entirely on developing and optimizing drugs aimed at inhibiting active HIV replication. Although this strategy has resulted in dramatic benefits for those with access to therapy, it has its limitations. Patients must take the drugs daily for life, and subtle toxicities accumulate over decades. Inflammation and immune dysfunction — which seem to have detrimental clinical consequences — persist even when viral replication is suppressed. Finally, and most importantly, the global resources necessary to deliver complex drug regimens, for many decades, to everyone in need are lacking. We require a therapeutic strategy in which a permanent disease-free state can be achieved after a more limited intervention. In other words, we need an effective cure for HIV infection1,2. On page 482 of this issue, Archin et al.3 report a proof-of-concept study that provides the first evidence that such a cure might one day be feasible.

HIV persists during effective therapy in part because its genome becomes stably integrated in certain white blood cells known as resting memory CD4+ T cells. These latent infected cells do not express viral proteins and hence remain invisible to the immune system. If activated, however, they can ignite new rounds of viral replication — a risk that forces patients to remain on therapy indefinitely. Theoretically, drugs that reverse latency might lead sequentially to HIV RNA synthesis, viral protein production, release of HIV particles and (hopefully) killing of the infected cell by the virus or by the patient’s immune system. Therefore, a cure might be possible if the latent virus in all infected cells can be forced out of its hiding place, leading ultimately to the death of the cells and to the elimination of the viral reservoir. Such a potential therapeutic approach is known as ‘shock and kill’ (Fig. 1).

Latency is maintained in part by the activity of histone deacetylase (HDAC), an enzyme that removes acetyl groups from DNA-bound histone proteins and, in so doing, affects gene expression. Although some HDAC inhibitors can induce mutations (at least in vitro), they might be able to reverse latency3,4,5.

Archin et al. set out to test the anti-latency activity of vorinostat, an HDAC inhibitor approved for the management of certain cancers. Given safety concerns, the researchers first screened patients to ensure that they had an HIV reservoir that was responsive to the drug. To do this, the authors extracted white blood cells from the patients (by a procedure known as leukapheresis), purified resting memory CD4+ T cells and then exposed such cells to the inhibitor. Of the 16 subjects screened, 11 exhibited a statistically significant vorinostat-mediated increase in HIV RNA expression and, of these, 8 patients eventually participated in the study.

The researchers administered a low dose of the drug (200 mg) to these eight subjects to ascertain tolerability. A few weeks later, a higher dose (400 mg) was given to determine anti-latency activity. Within six hours of this dose, the authors extracted resting memory CD4+ T cells from the patients to measure the concentration of cell-associated HIV RNA. In the eight subjects, levels of HIV RNA in resting CD4+ T cells increased in response to vorinostat, with the mean increase being 4.8-fold and the range 1.5–10.

As is common in such first-in-person clinical trials, this provocative study raises more questions than it answers. First, how should the field balance the ethical concerns about administering potentially toxic drugs to HIV-infected people who are otherwise healthy? The ideal population for these studies are those who have been doing well on long-term therapy, but this just happens to be the group with the lowest apparent need for a cure. Second, will future studies of anti-latency drugs require a costly and inconvenient leukapheresis before and after drug exposure? In San Francisco, the cost for such a procedure is over US$2,500.

Figure 1 | Getting HIV out of its hiding place. Current treatments for HIV infection limit replication of the virus but do not eradicate it, as the viral genome remains integrated into the DNA of some white blood cells (memory CD4+ T cells). Archin et al.3 tested the potential for a new therapeutic approach (‘shock and kill’) that involves activating viral replication. The authors show that treating HIV-infected patients with the drug vorinostat leads to activation of HIV genes, as revealed by an increase in the synthesis of viral RNA. A similar approach could be one day used to ‘awaken’ the dormant HIV in patients; the infected cells would then be killed either by the virus itself (not shown) or by the patient’s immune system. Moreover, current treatments such as highly active antiretroviral therapy would protect uninfected cells from becoming infected.
Therefore, a sensitive and high-throughput measure of viral latency is clearly needed.

Third, how much of the viral reservoir might be eliminated by HDAC inhibition? In the current study, the cells isolated from 5 of the 16 tested subjects failed to show any response to vorinostat and therefore these patients were not eligible for the clinical trial. Although those who eventually received the drug exhibited an increase in HIV RNA production, the overall clinical effect was probably marginal, as the concentration of viral particles in plasma did not increase and there was no apparent decrease in the size of the HIV reservoir.

Fourth, which assays will we use in the future to screen potential drug candidates for anti-latency activity? Vorinostat has demonstrated activity in most tests\(^6\), but not all\(^7\). Finally, what is the fate of the virus-producing cells after HDAC inhibition? Although many investigators have assumed that either the virus or the host immune system would destroy such cells and would therefore clear the virus, recent data suggest that this might not be true\(^8\). If the ultimate goal is to shock and kill infected cells, then more work is needed on how to eliminate them\(^9,10\).

Despite these uncertainties, the importance of Archin and colleagues’ study cannot be overstated, as it provides a rationale for an entirely new approach to the management of HIV infection. In many ways, their results are comparable to the initial finding that the anti-HIV drug zidovudine (also known as AZT) reduced viral replication in people. Even though zidovudine proved to have limited benefit on its own, the result showed that HIV could be inhibited, and the drug eventually became the basis for the first generation of drug combination regimens. I hope that HDAC inhibitors could ultimately become part of a combination approach to curing HIV infection.

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**The abilities of instabilities**

**ALI PASSIAN & THOMAS THUNDAT**

Despite the many advantages offered by nanoparticles as materials for a wide variety of applications, their use is currently limited by the lack of scalable, high-throughput techniques for synthesizing multifunctional particles of uniform size. Nanoparticles that are made from multiple materials are especially interesting, because they have potential applications in many devices, such as biological and chemical sensors. On page 463 of this issue, Kaufman et al.\(^1\) report a scalable fabrication technique that uses the dynamic instabilities of a fluid column to make particles of complex architecture\(^\ast\). Using this approach, they have prepared same-sized particles from combinations of vastly different materials, and with an unprecedented range of particle sizes.

Instabilities in fluid systems often produce undesirable effects — for example, standing up in a canoe can induce buoyancy instabilities that capsize the boat. However, instabilities can prove highly useful if they are harnessed properly, as Kaufman et al. demonstrate. Their fabrication technique depends on ‘Plateau–Rayleigh instabilities’ (PRIs), which are often manifested as remarkable flow patterns when a fluid is perturbed by heat.

Although the effects of PRIs can be quite subtle, these instabilities can grow exponentially, spatially and temporally modifying the motion or the flow pattern of a continuous body of fluid until it breaks up into ‘pieces’. This is what happens when droplets form from dripping taps. The resulting fluid domains tend to form as spheres, as demonstrated in the microgravity environments of space laboratories\(^\ast\). When small sinusoidal perturbations are engendered along a fluid column composed of different liquids, the wavelengths and growth rates of the perturbations depend on the fluid viscosities and on the diameters of the participating fluid columns — an effect exploited by Kaufman et al. in their technique.

The authors’ process for making particles begins with a ‘preform’ — a centimetre-scale rod in which a core of the materials to be made into particles is clad by a shell of scaffold material. This rod is heated and drawn into a fibre, until the diameter of the core matches the desired diameter of the particles. The fibre is then heated in such a way as to induce PRIs, which cause the core to split into spherical droplets (Fig. 1). These droplets are frozen in situ, forming particles encased within the scaffold material.

Microstructure—fabrication techniques in which PRIs break up multilayer, coaxial fluidic columns have been explored previously. For example, microspheres\(^3\) have been prepared using a microfluidic device in which droplets of a gel flowing at the core of a column of oil are polymerized by exposure to ultraviolet light. A similar approach\(^4\) has been used to fabricate microcapsules and strips of connected microcapsules. However, Kaufman and colleagues’ method differs by virtue of having achieved PRIs in prefabricated core–shell fibres. Most impressively, their technique is scalable, allows a variety of particle morphologies to be made (including ‘beach ball’ particles that contain six segments made of alternating materials), and works for particle sizes ranging from 20 nanometres to 2 millimetres — a whopping five orders of magnitude.

Other methods for making particles — based either on chemical synthesis\(^5\) or on physical methods such as laser ablation in liquids\(^6\) — have been used to generate suspensions of a variety of highly pure nanoparticles. However, a great advantage of Kaufman and colleagues’ technique is its ability to create multilayered nanoparticles.

Understanding and controlling fluid behaviour at a small scale is of tremendous technological importance. The Navier–Stokes equation is used to describe the motion of fluids, but this must often be modified to take into account the constraints of specific flow systems. For example, a study\(^7\) of the de-wetting of thin polymer films has shown that thermal noise — random forces in a fluid caused by molecular motion that is induced by thermal agitation — can strongly influence the time- and length-scales of the film’s flow. Given that Kaufman et al. use thermal perturbation as a means to induce instabilities, and that thermal noise increases with temperature, one would expect the break-up of the thin liquid columns in their technique to be influenced by such