enough strain energy to overcome the weak bonds between the film and the strontium ruthenate.

Kum and co-workers demonstrated the transferral of oxide membranes to other materials for: strontium titanate, yttrium iron garnet and magnetic cobalt ferrite, produced by pulsed-laser deposition; lead magnesium niobate–lead titanate (PMN-PT), formed by sputtering; and ferroelectric barium titanate, made by a process called molecular-beam epitaxy. One example of a stacked structure produced by such transferral consists of a 300-nm-thick layer of cobalt ferrite, an electrode and a 500-nm-thick layer of PMN-PT (Fig. 1c).

The authors found that this structure displays high magnetostriction (coupling between magnetic and mechanical behaviour) and piezoelectricity (coupling between electric and mechanical behaviour), because it is free-standing rather than being clamped by a substrate. Cobalt ferrite, PMN-PT and yttrium iron garnet have different crystal structures, making it difficult to stack these materials by the usual growth scheme without such clamping.

Kum *et al.* also stacked graphene and oxide membranes to examine the electrical coupling between these materials. The density of electric charge in graphene can be inferred from the positions of peaks in Raman spectra — spectra generated through the scattering of incident light. The authors found that these positions depend on the stacked structure, indicating that charge is transferred across graphene–membrane interfaces. These results suggest that stacks of other combinations of materials will offer ways to integrate the various functions of oxides with mature device technologies.

The authors' exfoliation technique enables complex-oxide films to be easily transferred from an epitaxial interface to any material. Because the thickness and stacking of films can be controlled, ultrathin membranes and stacks of various membranes could be possible. Such a simple way of transferring the functions of oxides might advance the field of oxide-based electronics through integration with emerging quantum material systems[10]. However, the availability of graphene-coated substrates could be a key issue for developing the method.

This technique will probably be extended beyond the transferral of complex-oxide films. For example, it might provide an innovative strategy for engineering interfaces, by allowing 2D or 3D films and membranes to be integrated with each other through effects associated with multiple couplings between them. An understanding of the chemical or physical bonds at the interface between membranes in stacked structures is crucial and will reveal how such an interface differs from the epitaxial one. Finally, unusual material combinations (in which, for example, the size and orientation of crystal lattices of membranes are mismatched) could enable useful interface functions that are difficult to achieve or control at the epitaxial interface.

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Virology

Latent HIV-1 gets a shock

Mathias Lichterfeld

HIV-1 can evade the immune system by hiding out in a dormant form. Two studies describe interventions that can effectively reactivate the latent virus in animals, potentially rendering it vulnerable to immune-mediated death. **See p.154 & p.160**

'Shock and kill' might sound like a military strategy, but in fact it describes the dominant model currently used in the search for a cure for HIV-1 infection. Although antiretroviral therapy (ART) is highly effective at limiting the extent of the infection, the virus can hide out in a 'latent' form in immune cells called $CD4^+$ T cells, undergoing little or no transcription and thus remaining undetected by the immune system[1,2]. When ART is stopped, these viral-reservoir cells can rapidly fuel HIV rebound. The theory behind 'shock and kill' involves the use of drugs that reverse this latency and could increase viral gene expression (shock), rendering the viral-reservoir cells vulnerable to elimination (kill) by other cells of the immune system. Two groups[3,4] now describe distinct interventions in animal models that cause what seem to be the most robust and reproducible disruptions of viral latency reported so far.

"The current studies showcase some of the conceptual and technical challenges intrinsically associated with pharmacological latency reversal."

In the first study, Nixon *et al.*[3] (page 160) focus on a drug called AZD5582, which can activate the transcription factor NF-κB — a major instigator of HIV-1-gene expression. AZD5582 was originally developed to treat cancer, and activates the 'non-canonical' NF-κB pathway, which results in an atypical type of NF-κB-driven transcription that is slow but persistent. The authors tested AZD5582 in two animal models: 'humanized' mice (which carry human-derived liver, bone-marrow and thymus cells) that were infected with HIV; and rhesus macaques infected with the HIV-related simian immunodeficiency virus (SIV). Both groups of animals were already receiving ART.

The authors demonstrated that AZD5582 treatment led to marked increases in the levels of viral RNA in $CD4^+$ T cells in a range of tissues in both species, indicating that transcription of the virus had been activated. This was combined with a substantial rise in virus levels in the blood. AZD5582 is not optimized for use in humans; nonetheless, these results suggest that pharmacological activation of the non-canonical NF-κB pathway could be an attractive way to trigger HIV-1-gene expression as part of a shock-and-kill approach (Fig. 1).

In the second study, McBrien *et al.*[4] (page 154) used an entirely different, though complementary, approach to disrupting viral latency. Again, the authors used both ART-treated humanized mice infected with HIV-1 and ART-treated, SIV-infected rhesus macaques. They combined two immunological interventions. The first involves antibody-mediated depletion of $CD8^+$ T cells — immune cells previously shown to act in concert with ART to reduce levels of viral transcription[5]. The second, administered concurrently, involves treatment with a drug called N-803, which strongly activates the signalling

molecule interleukin-15 (IL-15), and which has been previously shown[6] to activate HIV-1 transcription *in vitro*. Like Nixon and colleagues, the researchers found that their treatment caused substantial increases in virus levels in the blood, and in viral RNA in cells from various tissues.

At first glance, the combined interventions used by McBrien and colleagues might seem contradictory, because IL-15 is one of the strongest activators of $CD8^+$ T cells[7,8]. But the synergistic effects of these two interventions raise the provocative possibility that the best strategies for targeting viral-reservoir cells involve a mix of immune interventions — suppressing immune components that seem to have a role in stabilizing viral latency (such as $CD8^+$ T cells) while activating others that can effectively disrupt latency (such as IL-15 signalling).

How exactly $CD8^+$ T-cell depletion interacts with IL-15 to reverse HIV-1 latency is unknown. Given the vast array of direct and indirect effects resulting from depletion of $CD8^+$ T cells[9], it will not be easy to define the precise molecular mechanisms underlying this synergy. But an understanding of this relationship might reveal downstream proteins that are jointly targeted by these interventions and that could therefore be used to optimize latency reversal in the clinic.

In addition to the advances they make, the current studies showcase some of the conceptual and technical challenges intrinsically associated with pharmacological latency reversal. First, the latency-reversing agents (LRAs) evaluated (as well as all other LRAs described so far[10]) target factors that have crucial roles in modulating host-cell gene transcription, in addition to viral transcription. Their use therefore comes with an intrinsic risk of toxic off-target effects. The toxicity of the LRAs described by McBrien *et al.* and Nixon *et al.* seems to be acceptable in animal models, with most showing no clinical side effects. However, much more stringent safety standards must be met in human clinical trials.

Mechanisms of viral latency might vary between individual viral-reservoir cells and are likely to be influenced by the position at which the HIV-1 genomes have integrated into the host-cell chromosomes[11]. It is therefore possible that only subsets of cells will respond to individual LRAs, which typically target one specific mechanism of viral latency. The actual proportion of viral-reservoir cells that responded to the interventions in the two current studies is uncertain, and would be difficult to determine experimentally[12].

Another uncertainty is how much of the increase in HIV-1 RNA is attributable to $CD4^+$ T cells carrying HIV-1 that can replicate effectively[13,14]. This is of interest because most viral-reservoir cells harbour HIV-1 genomes that contain lethal sequence defects, probably as a result of errors introduced during reverse transcription of viral RNA, which produces the viral DNA that is integrated into the host genome. These defective viral genomes can often still be transcribed and respond to LRAs, but they cannot cause viral rebound when ART is stopped and so do not represent the main target for shock-and-kill interventions. In addition, it is unclear how disrupting latency might influence the evolutionary dynamics of the reservoir cells — whether, for instance, a shock treatment kills some subsets of $CD4^+$ T cells that are highly susceptible to latency disruption, but confers a selective advantage on other subsets of non-susceptible, difficult-to-reactivate cells.

Most importantly, neither of the interventions tested in the current studies led to a change in the expression of markers of viral-reservoir size. A decrease in these markers is the most informative and crucial endpoint parameter for shock-and-kill approaches. The absence of an effect on viral-reservoir size probably reflects the fact that the studies were mainly designed to investigate latency reversal, and lacked dedicated 'kill' interventions. Combining 'shock' interventions with 'kill' components is a key next step. In fact, that they provide a suitable model for evaluating 'kill' strategies in the setting of robust and efficient latency reversal might be one of the strengths of the current studies.

Finally, the work of Nixon and colleagues and McBrien and colleagues should not distract from the fact that the shock-and-kill strategy currently remains largely a theoretical concept, not a therapeutic reality. Establishing evidence for its ability to reduce viral reservoirs and to deliver real benefits to patients will require much more work.

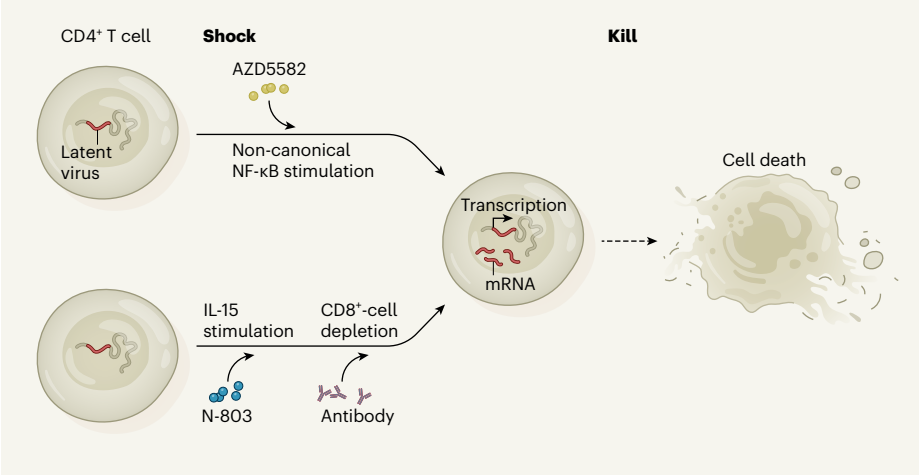

Figure 1 | Two approaches to reactivating dormant HIV-1. HIV-1 can integrate into the genome of $CD4^+$ T cells in a latent form — it is not transcribed into messenger RNA and so is not detected by the body's immune system. Two papers describe 'shock' treatments that can reactivate transcription of latent HIV in mice and the related virus SIV in monkeys. Nixon *et al.*[3] used a drug called AZD5582 to activate the non-canonical NF-κB signalling pathway, which stimulates virus transcription. McBrien *et al.*[4] used two interventions — a drug called N-803 to stimulate the protein IL-15, which promotes transcription, and an antibody treatment that depletes immune cells called $CD8^+$ T cells, which seem to have a role in dampening HIV transcription. After these shock treatments have reactivated the virus, interventions that target and kill the virus-carrying $CD4^+$ T cells should help to eliminate the latent viral reservoir. Such treatments remain to be designed.

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This article was published online on 22 January 2020.