Functional cure of HIV: the scale of the challenge

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Abstract | A variety of interventions to induce a functional cure of HIV are being explored, with the aim being to allow patients to cease antiretroviral therapy (ART) for prolonged periods of time or for life. These interventions share the goal of inducing ART-free remission from HIV pathogenesis and disease progression but achieve this in quite different ways, by reducing the size of the latent reservoir (for example, small-molecule stimulation of latently infected cells), reducing the number of target cells available for the virus (for example, gene therapy) or improving immune responses (for example, active or passive immunotherapy). Here, we consider a number of these alternative strategies for inducing post-treatment control of HIV and use mathematical modelling to predict the scale of the challenge inherent in these different approaches. For many approaches, over 99.9% efficacy will likely be required to induce durable ART-free remissions. The efficacy of individual approaches is currently far below what we predict will be necessary, and new technologies to achieve lifelong functional cure are needed.

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Current antiretroviral therapy (ART) is highly effective. It suppresses plasma viral RNA to below the standard level of detection for prolonged periods of time and halts viral evolution. However, in most patients, the virus rapidly rebounds within weeks of ART interruption[1,2] (FIG. 1a). Several therapeutic approaches that aim to prevent or delay viral rebound after treatment interruption, producing a post-treatment remission or functional cure of HIV, are being investigated. These would allow subjects to cease ART for prolonged periods, reducing side effects, cost, the emergence of drug resistance and stigmatization. Current interventions primarily focus on driving the reactivation of latent virus, the targeting and destruction of highly infected cell subsets, or the use of gene therapy to either target the latent provirus or make cells refractory to viral replication. In addition, immunotherapy and vaccination are being explored to prevent HIV reactivation or to reduce post-reactivation viraemia and keep it at very low levels (FIG. 1b–d). Inherent to each approach is the requirement to remove a sizeable proportion of latently infected cells or control the virus at a particular level. Achieving some level of remission seems feasible, as there are a number of case studies in the literature of patients who have experienced prolonged post-treatment remission or viral control[3–5]. However, in almost all these cases, the virus rebounded in a shorter time frame than would be acceptable as part of a functional cure for HIV. Therefore, it is important to consider what level of efficacy would be required from each treatment methodology in order to achieve an acceptable functional cure. Here, we use a mathematical modelling framework to analyse and compare the predicted impacts of different potential interventions (outlined in Supplementary methods). For each intervention, we estimate the magnitude of the therapeutic effect that would be required to produce a functional cure of HIV of sufficient scale and durability to contribute to global goals for reducing HIV transmission and disease.

Shrinking the latent reservoir

One approach to producing prolonged post-treatment control is to either eliminate or shrink the HIV reservoir down to a size where successful viral reactivation from latency occurs rarely, if ever[6]. This approach appears to be the mechanism of delayed viral rebound observed in the so-called Boston patients, who underwent bone marrow transplantation while on ART. The pretransplant conditioning removed the majority of T cells (including latently infected cells) and replaced them with (uninfected) donor cells[3]. However, a small residual HIV reservoir persisted, leading to a very delayed production of replication-competent virus and viral rebound after only 3–7 months of remission[3]. A similar phenomenon was observed in the so-called Mississippi baby case, in which it is thought that very early treatment and a very low initial viral reservoir size allowed a prolonged period of nearly 2 years of treatment-free remission before viral reactivation led to rapid viral replication[4]. Extremely early treatment of adult infection has also

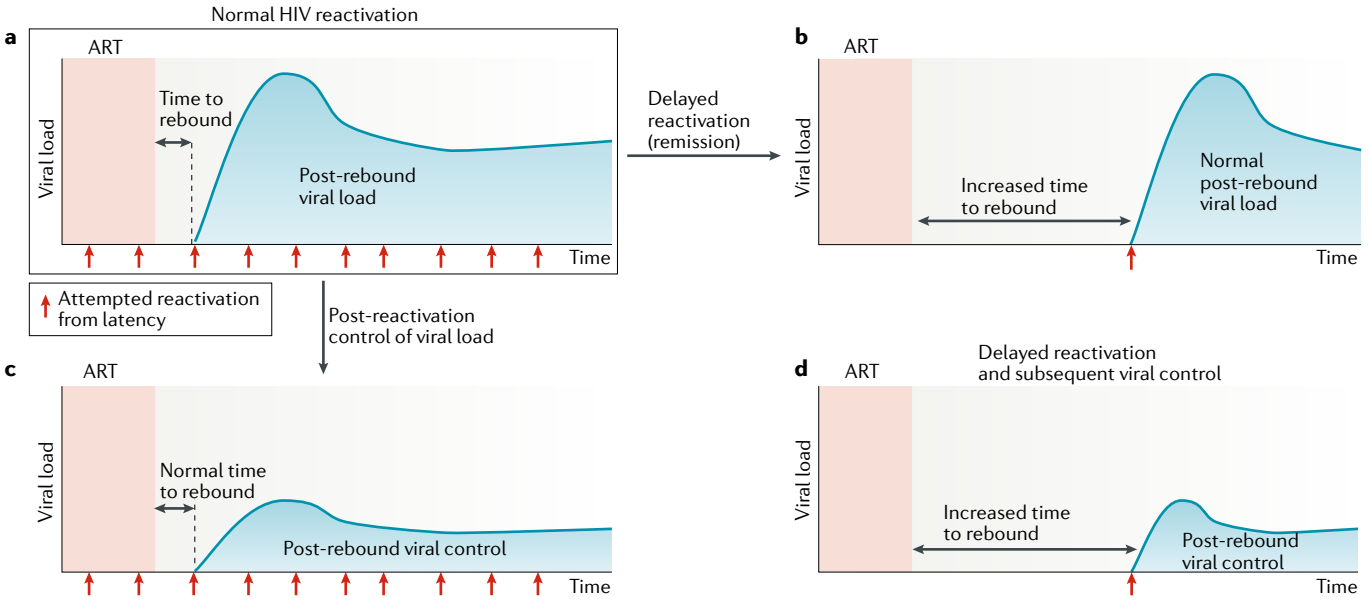


Fig. 1 | **Potential effects of interventions to control latency. a** | Following treatment interruption, HIV typically rebounds within a few weeks, reaching set point viral loads similar to pretreatment levels. **b** | Treatments that decrease the frequency of successful HIV reactivation from latency will delay the time to rebound. However, once viral recrudescence occurs, viral loads reach the same levels as depicted in part **a**. **c** | Interventions may also act to promote post-rebound control of the viral load, allowing low levels of viral replication but preventing disease progression. **d** | Combined therapies may also mediate both delayed rebound and post-rebound control if rebound does occur. ART, antiretroviral therapy.

been associated with a very small reservoir size; however, it correlates with a much more modest prolongation of ART-free remission of only days or weeks[7,8].

These case studies suggest that shrinking the reservoir can induce prolonged remission. But how much of a reduction in reservoir size is required to achieve a clinically useful period of remission (see BOX 1)? Successful HIV reactivation from latency after treatment interruption is estimated to occur with a frequency of approximately once a week in most patients treated during chronic infection[1]. This estimation gives us a scale against which to assess how much we need to reduce the normal frequency of HIV reactivation to produce a post-treatment remission of different duration[6]. For example, a 20-fold reduction in the frequency of reactivation should result in an average remission duration of 20 weeks (similar to that observed with bone marrow transplantation[3]); a 100-fold reduction should result in a 2-year remission (similar to that seen in the Mississippi baby case report[4]); and a 1,000-fold reduction would be required for an average remission duration of 20 years. Thus, a reduction of somewhere in the order of 1,000-fold in the frequency of reactivation would be necessary to produce a meaningful period of remission, in which the threat of viral rebound, immunodeficiency and possible onward transmission would not occur in the majority of patients for many years (discussed in more detail in REF.[6]). Importantly, the key metric we discuss here is the frequency of reactivation, not the number of latently infected cells per se (see BOX 1). For approaches aimed at reducing the size of the reservoir, an average 20-year remission equates to at least an average 99.9% reduction in the frequency of reactivation.

Inducing activation and death of latent virus. One approach to reducing the frequency of HIV reactivation is to induce the activation of latent virus, leading to the spontaneous or immune-mediated removal of infected cells[9]. To date, the effects of short-term treatment have been modest[10–12], with modelling suggesting that application of a single treatment or a combination of a few current-generation treatments may at best remove a few per cent of the reservoir[13]. Although it seems unlikely that a single treatment could reduce the frequency by >99% (as would be required), multiple rounds of treatment could have a more profound effect over time, assuming that re-treatments cumulatively reduce the frequency of reactivation (FIG. 2a,b). Thus, for example, a treatment that was capable of reducing the reactivation frequency by 50% upon each treatment could potentially be administered twice to reduce the frequency to one-quarter of its original rate, once again to reduce it to one-eighth, and so on. Such a treatment would need to be administered 10 times to reduce the reactivation frequency 1,000-fold, thus achieving the average 20-year remission discussed above. If, however, the effect of the treatment was more modest, with a 20% reduction per treatment cycle, this would mean that the treatment would need to be given ~30 times, while an intervention that reduced reactivation by 10% per cycle would need to be administered ~65 times. It quickly becomes clear that not only the potency but also the tolerability of the treatment is important.

Another major consideration is whether an intervention continues to retain its efficacy over multiple rounds of treatment. Indeed, if we have a treatment whose efficacy wanes by 5% with each round of treatment, and if

Box 1 | **Measuring reservoir versus measuring reactivation frequency and remission?**

A major question is what to measure to predict the effectiveness of anti-latency therapy. A variety of methodologies have been developed that try to determine the size of the latent reservoir in individuals with HIV on treatment. These methodologies range from strategies to measure total HIV DNA, replication-competent DNA and cell-associated RNA to assays that measure HIV reactivation following latent cell stimulation in vitro[57,58]. These methods are typically performed on peripheral blood rather than tissue samples because of ease of access. In general, these different measures of the reservoir often do not correlate well with each other and have proved only weakly predictive of the time to viral recrudescence after treatment interruption[8,59,60]. Thus, at present, there is no clear agreement on which reservoir we should be measuring. The frequency of reactivation from the reservoir will be affected by both the number of latently infected cells (harbouring replication-competent virus) and the probability of reactivation per cell. Heterogeneity in latent cell behaviour can play a major role in this. For example, not all replication-competent HIV DNA may be equally prone to reactivation, depending on the integration site, latent cell phenotype or anatomical location of the latently infected cell. Therefore, measuring the number of latent cells in peripheral blood or even the ability of these cells to be reactivated in vitro may not capture the potential contribution of different tissue sites of latency to the frequency of latent viral reactivation. For this reason, eliminating subsets of latently infected cells can have a greater or lesser effect on the overall frequency of reactivation, depending on whether the subset has a higher or lower than average level of infection and reactivation frequency per cell. Simply measuring changes in HIV DNA or in vitro reactivation (using circulating cells) before and after treatment may be a poor surrogate of the effects of treatment on the frequency of reactivation and on remission.

If post-antiretroviral treatment interruption remission is the goal, why not measure it directly?
Measuring the reduction in levels of circulating HIV DNA, RNA or in vitro viral reactivation from peripheral blood mononuclear cells may be poorly predictive of changes in the duration of remission, as recent studies suggest that even patients with low or undetectable levels have near-normal time to detection of the virus after antiretroviral treatment interruption (ATI)[8,30]. In this Analysis, we discuss the effects of therapy on reducing the frequency of reactivation rather than reducing the reservoir size, as the average frequency of reactivation is directly related to the expected duration of remission[1,6,53,61]. Thus, we do not speculate whether, for example, halving the level of circulating HIV DNA also halves the frequency of reactivation or has a greater or lesser effect on reactivation (for example, if it preferentially removes the most or least reactivation-prone cells). Because the goal of remission is to delay the time to viral rebound, we propose that directly measuring post-antiretroviral therapy time to detection or frequency of reactivation of a virus is the best way to measure our progress towards successful HIV remission[1,6,62].

Predicting the effectiveness of anti-latency interventions
It is generally assumed that reactivation from latency is a random process, with reactivation and viral recrudescence occurring at some average frequency. Remission from viral rebound is the time between treatment interruption and the detection of the virus[1,53]. Early estimates of the frequency of reactivation relied upon theoretical models of the time to drug resistance under therapy and estimated rates of approximately four reactivations per day[53,63,64]. More recently, the frequency of HIV reactivation has been estimated directly from an analysis of data on the time to recrudescence after treatment interruption in patients and was found to be approximately once a week in HIV[1,64]. Increasing this delay between reactivation events (decreasing the frequency of reactivation) will increase the time from treatment interruption to viral recrudescence.

the aim is to achieve a 99.9% reduction in reactivation in less than 100 rounds of treatment, this would be achievable only if the efficacy in the first round was at least 32%. Additionally, the maximum level of reduction in frequency ever achievable by the treatment is also limited. For example, if a treatment that began with an ability to reduce reactivation size by 30% became 5% less effective upon each subsequent treatment (that is, it achieves a 30% reduction in reactivation upon the first treatment, 28.5% upon the second, 27.08% upon the third, and so on), then we find that the maximum remission that could ever be achieved is 13.3 years (see FIG. 2c). Thus, three aspects are crucial to predicting the effectiveness of therapies aimed at reducing reservoir size and the frequency of HIV reactivation: potency, tolerability and sustainability of the treatment effect over multiple rounds.

Targeting cell subsets. A minimally toxic approach to reservoir reduction would be to selectively eliminate latently infected cells, leaving the uninfected cells intact. However, the silent nature of latent virus makes it difficult to identify these cells. A number of studies have compared the levels of provirus or reactivation-competent virus in different cell subsets and identified subsets that have higher levels of infection[14]. However, critically, it is not the increased level of infection in the targeted subset that is important but the proportion of total latent virus (or more specifically, total number of reactivations; see BOX 1) that occurs within the subset. For example, if a subset is 10 times more infected than other cells but comprises only 5% of the total number of infected cells, then it still represents only approximately 35% of the total number of infected cells. Even if we could target and entirely remove this subset, only 35% of the latently infected cells would be removed, and a durable remission would not be achieved. We would still need to deal with the remaining 65% of latent virus that is resident in non-targeted cells. The frequency of reactivation in the non-targeted subsets would still need to be reduced by approximately 99.8% to achieve an overall 1,000-fold reduction in reservoir size. Even complete eradication of a subset can have a relatively small benefit in reducing the overall frequency of reactivation or inducing notable remission unless this is also combined with a concomitant reduction in the frequency of reactivation in the general (non-subset) HIV reservoir.

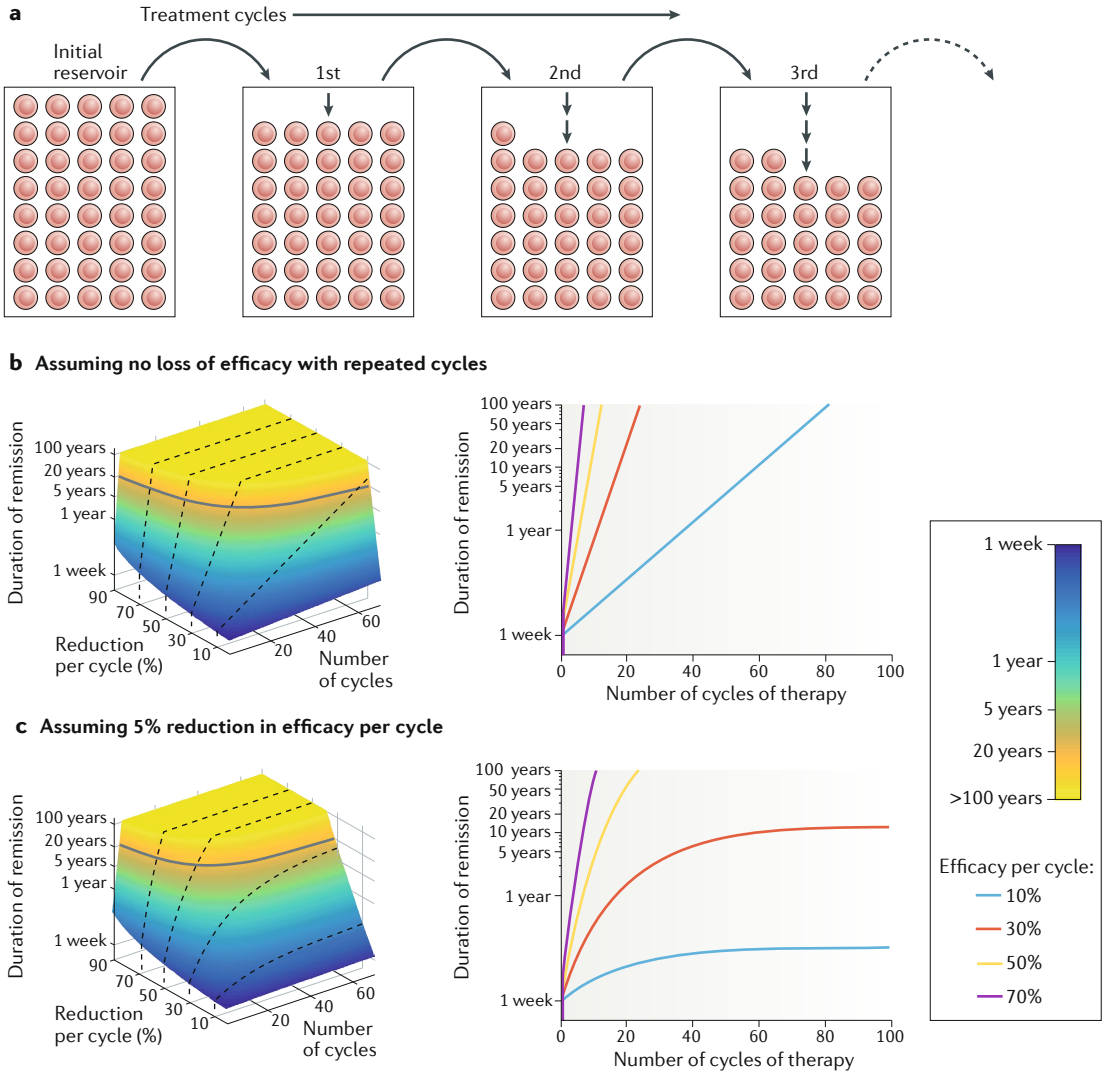


Fig. 2 | **Predicting the effects of reducing the reservoir or the frequency of reactivation on the duration of remission.** **a**,**b** | For a treatment that reduces the average frequency of reactivation by a given percentage, we can estimate the average duration of remission that may be expected if that treatment was given for a specified number of cycles. The number of treatment cycles required to achieve a particular duration of remission is indicated for treatments that reduce the frequency of virus reactivation by 10%, 30%, 50% and 70% upon each cycle (when repeated treatments remain equally effective). For example, a treatment that reduces the frequency of virus reactivation by 30% each cycle must be given 20 times to achieve a predicted average 20-year remission. **c** | When repeated treatment shows a waning effect (in this case, a 5% reduction in efficacy per cycle), more treatment cycles are required. Treatments with an initial efficacy >32% would then be required to achieve a 20-year remission in < 100 treatment cycles.

Targeted gene silencing. A prerequisite for latent cell reactivation is the presence of an intact HIV genome, direct targeting of these genomes is one avenue to reducing the frequency of reactivation. The use of CRISPR–Cas9-based gene therapy to cleave provirus and thus eliminate latent HIV or to silence HIV reactivation has been suggested. Currently, these technologies can reduce viral replication by 10–20-fold in vitro and by 10-fold in vivo in animal models[15–18]. However, the problem of scale is also an issue here. By extension from the analysis above, if, for example, a gene therapy aims to enter and cleave or silence HIV proviral DNA, it must aim to enter 99.9% of infected cells to silence a meaningful proportion of the reservoir (FIG. 3a,b). This level of gene delivery is currently not achievable. For example, ex vivo transduction of human $CD4^+$ T cells with an adeno-associated virus encoding zinc-finger nucleases targeted at CCR5, a co-receptor used by HIV for cell entry, achieved efficacy rates of less than 30%[19]. While higher levels of transduction of $CD34^+$ haematopoietic stem cells have been reported (>50%), there are issues with achieving efficient engraftment of substantial numbers of these cells.

Removing susceptible HIV targets

Another example of prolonged antiretroviral-free remission from HIV infection is the Berlin patient[20]. In this case study, the patient received a bone marrow transplant with CCR5-deficient bone marrow. Thus, the transplant conditioning acted to remove latently

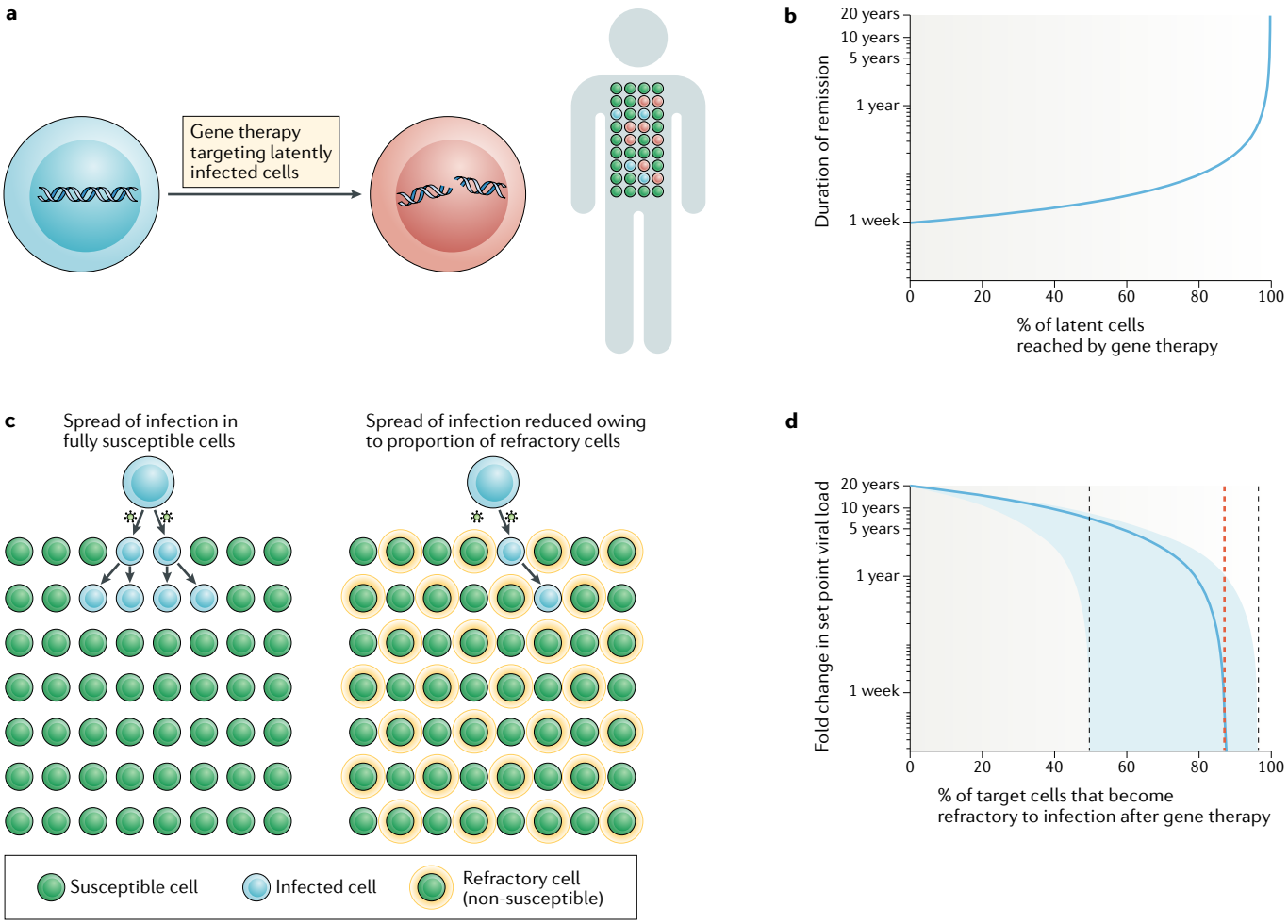


Fig. 3 | **Modelling the impact of gene therapy on HIV remission. a** | If gene therapy is used to target and inactivate latent HIV provirus from infected cells, then the average length of remission achieved will be directly related to the proportion of infected cells that can be treated by gene therapy. **b** | The relationship between the number of cells effectively treated (proportion of provirus inactivated) and the duration of remission is shown. If gene therapy could reach 90% of cells, it would induce an average of 2.5 months (10 weeks) of remission. **c** | If gene therapy can render the transduced cells permanently resistant to further HIV infection, then it will also reduce the number of uninfected cells that are susceptible to HIV infection. **d** | By reducing the number of target cells for infection, viral replication and the set point viral load achieved after HIV rebound will be reduced. Modelling suggests that if enough target cells are made refractory to infection, then the ability of HIV to replicate at all should be effectively blocked (as was observed in the case of CCR5-defective bone marrow transplantation[20]). Current estimates are that it would be necessary to make >87.5% of cells resistant to infection to block viral replication (red dashed line, assuming a baseline basal reproductive ratio of HIV $R_0 = 8$). For different levels of viral infectivity, the proportion of cells required to become resistant might vary from 50% to 97% (shaded blue area, for R_0 values between 2 and 32).

infected recipient cells (reducing the size of the reservoir), and the transplant itself replaced susceptible recipient ($CCR5^+$) $CD4^+$ T cells with resistant ($CCR5^-$) donor cells, thus reducing the ability of the virus to replicate. It is thought that transplantation with $CCR5^-$ bone marrow effectively removes susceptible $CD4^+$ T cells, and so even if the virus reactivates from latency, it has very few susceptible $CCR5^+$ $CD4^+$ T cells in which to replicate. This transplantation has provided more durable control than a bone marrow transplant alone, as no rebound has been observed in more than 10 years.

This example raises an alternative use for gene therapy: if the HIV-suppressive effect of gene therapy is permanent (making the transduced cells and subsequent daughter cells refractory to future infection), this may not only reduce the frequency of reactivation but also have the additional effect of removing susceptible target cells for future viral replication (FIG. 3c). Importantly, the removal of susceptible cells may actually present a greater benefit from gene therapy than the reduction in the viral reservoir. Modelling suggests that if it were possible to protect 50% of cells from infection (that is, 50% chimerism with gene-modified resistant cells), then set point viral loads would be reduced by approximately 60%[21,22] (see Supplementary methods), which is consistent with anecdotal evidence in human and animal infection[19,23,24]. However, if 75% of cells were protected, then the viral load would be reduced to 14.3% of its normal level. Importantly, we can show that there is a minimum number of susceptible cells required for the virus to grow

at all (once the number of target cells is below a threshold, each currently infected cell is able to infect only less than one additional cell, and the virus essentially peters out; FIG. 3c). Our modelling suggests that if >87.5% of cells were made refractory to infection, then the virus would be unable to grow successfully (assuming a basal reproductive ratio of HIV $R_0 = 8$)[22] (FIG. 3d).

Thus, three major factors to consider in any gene therapy approach are the transduction efficiency (proportion of cells that can be reached), the proportion of these cells that then engraft and are long lived or capable of generating progeny and to what extent the treatment makes cells durably resistant to future infection.

Immune control of HIV

Immune recognition of the virus or infected cells may also contribute to long-term, post-treatment HIV remission. This may occur in a variety of ways: immune recognition may help to shrink the viral reservoir, or immunity may be 'reactivation-blocking' in that it acts to block or control a proportion of reactivation events, reducing the frequency of successful HIV reactivation events and delaying the time to viral recrudescence. Alternatively, initial viral reactivation from latency may occur, but immunity may be 'reactivation-controlling', and viral replication may subsequently be controlled such that it remains at low levels. Studies of cohorts of patients who received ART very early in primary HIV infection have shown that a subset of patients exhibits varying degrees of post-treatment control[25,26]. Analyses of the viral dynamics after treatment interruption suggest that both reactivation-blocking and replication-controlling effects may have occurred in these cohorts, as most patients did show early evidence of viral recrudescence, followed by a period of immune control[25,26]. This control was attributed to an alteration in the viral–immune balance as a result of early treatment, in which host immunity may be induced in primary infection and is not impaired by ongoing viral replication if primary HIV infection is rapidly controlled by ART[27,28].

However, other studies of patients treated early in infection have not always shown similar control of HIV replication[29,30], perhaps because of the importance of the timing of initial treatment. If the treatment is initiated too early, the very small amount of antigen exposure means that little host immunity will be induced[31,32]. If the treatment is started too late, then host immunity may be exhausted[33] or eliminated by the infection[34,35], or viral escape mutation may have occurred[36]. As the majority of patients undergoing ART are not identified and treated in early infection, early initiation of ART will be of limited value for the immune control of HIV in most subjects.

Immune targeting of the reservoir. The latent reservoir of HIV is generally thought to be invisible to immune surveillance, as truly quiescent latent cells are not thought to express notable amounts of viral antigen. However, a proportion of latently infected cells may transiently express viral antigens periodically (as a result of cell division or activation), rendering them susceptible to immune killing by $CD8^+$ T cells or antibody-dependent mechanisms. Immunization or passive immunotherapy during ART might induce responses capable of killing cells during these periods of antigen expression, allowing the elimination of a proportion of the reservoir each year (depending on the frequency of antigen expression)[37,38] (FIG. 4a). If, for example, 10% of latently infected cells expressed antigen each year, and an effective immune response could remove these cells, then the reservoir could be reduced by 10% per year, and we would see a 90% reduction over 20 years (producing an average 10-week remission). However, if 50% of cells expressed antigen each year, then after 10 years the reservoir would be reduced to 0.1% of its starting value, achieving an average 20-year remission (FIG. 4b). It is clear that this requires both a fairly high proportion of cells to express antigen regularly and the efficient killing of these cells in order to contribute to notable remission.

Therapeutic vaccination to prevent HIV reactivation. Vaccination or immunotherapy during ART offers the potential of enhanced immune control during treatment interruption either to prevent viral recrudescence or to control the rebound virus and limit it to low levels. HIV reactivation from latency after treatment interruption might be thought of as similar to a serial low-dose challenge by a reactivating virus. Studies suggest that, on average, a latently infected cell successfully initiates viral recrudescence approximately once every week following treatment interruption[1]. Thus, if we think of vaccine efficacy as the proportion of these reactivation events that are successfully blocked, then we can estimate the expected delay in reactivation we would obtain for different levels of vaccine efficacy (FIG. 4c,d). Vaccination studies in macaques infected with simian immunodeficiency virus (SIV) demonstrated significant protection against serial challenge, blocking as much as 80% of challenges[39]. However, the most successful current HIV vaccine trial showed only approximately 30% efficacy against sexual transmission[40]. Passive immunotherapy in macaques with SIV has also been shown to prevent multiple low-dose challenges[41,42], and passive immunotherapy during HIV treatment interruption has shown evidence for delays in the time to detection of a virus[43,44]. FIGURE 4e shows the relationship between vaccine efficacy (that is, the proportion of HIV challenges to HIV reactivations from latency that are blocked) and the predicted average duration of post-treatment remission. We see that even a vaccine that is capable of blocking 80% of reactivation events would still allow most patients to have a breakthrough reactivation event within 5 weeks. FIGURE 4e illustrates that vaccines or immunotherapies capable of blocking >99% of reactivation events would be required to produce clinically significant delays in the time to detection of a virus.

Immune control of HIV following viral recrudescence. An alternative strategy to achieve HIV remission after ART interruption may be to induce post-treatment immune control of HIV after the virus has reactivated. That is, even if the immune system is unable to block HIV recrudescence, it may still be able to control viral replication and keep it at low levels, as sometimes observed in patients treated early after infection[25,26].

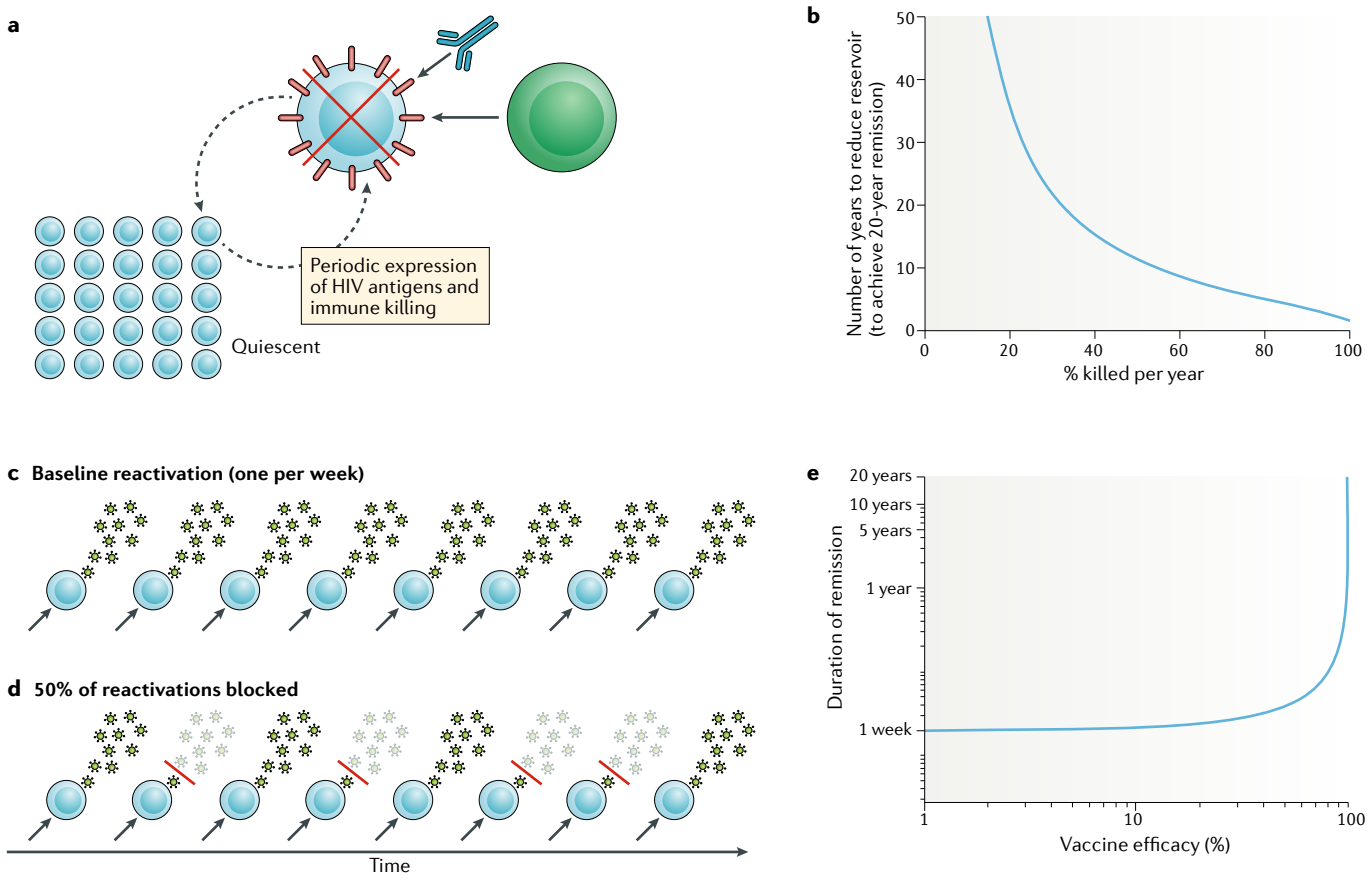


Fig. 4 | **Effects of immune therapies on HIV remission.** Vaccines or immunotherapies may delay HIV reactivation either by reducing the size of the reservoir or by blocking HIV reactivation events. **a** | Latent cells may periodically express HIV antigens and thus be susceptible to immune killing. **b** | Depending on the proportion of cells that express antigen and are killed each year, it will take different amounts of time until the reservoir is notably reduced. **c**,**d** | If vaccination or passive immunotherapy is able to block a proportion of HIV reactivation events, then it may delay the time to viral recrudescence. **e** | For a given vaccine efficacy (proportion of infections blocked, as shown on the *x* axis), the expected average duration of remission can be estimated (*y* axis).

It seems likely that if sufficient immune protection is present at the time of HIV reactivation, this may switch the host–viral balance in favour of host control[28,44–46]. Inducing such disease-modifying immunity has proved challenging in the context of prophylactic HIV vaccine development, in which the only successful prophylactic vaccine did not alter set point viral loads after infection[40]. However, this process may be easier in the context of therapeutic vaccination and treatment interruption, where the timing of treatment interruption can be manipulated to coincide with peak immunity or the presence of passive antibodies[44–46]. The effects of early ART on long-term viral control suggest that shifting the balance towards host control of viral replication is possible in this context[26,47]. Such post-recrudescence control offers the potential for long-term ART-free remission. However, it is not known whether such immune control can be reliably induced in patients with HIV treated during chronic infection or whether this control can be maintained for prolonged periods or might be undermined by factors such as immune escape[47–49]. Another major question is what level of viral control would be deemed sufficient. That is, at what level of ongoing viral replication would the trade-off between ongoing ART and the risks of disease progression and transmission balance out? Recent studies have suggested that even in patients with very low viral levels (>200 copies per ml), the risk of disease progression is increased compared with that in patients with an undetectable virus (<50 copies per ml)[50]. Transmission risk is thought to be eliminated at viral loads <200 copies per ml[51,52]. Together, this suggests that a decision to withhold ART would likely require post-treatment control of viral loads to low levels (at least <200 copies per ml).

Combinations and synergies

Given the diversity of strategies discussed above, combinations of two or more therapies clearly have the potential to increase the overall effect of treatment. An obvious opportunity for combination therapy lies in combining agents that act in a similar way to achieve greater potency (FIG. 5a, left panel). Thus, for example, treatments inducing the activation and death of latently infected cells, strategies targeting subsets of infected cells, gene therapy to remove provirus and inducing immunity to block HIV reactivation all act to reduce the frequency of reactivation, and we might expect their effects to be additive (FIG. 5). However, each agent in a combination would still

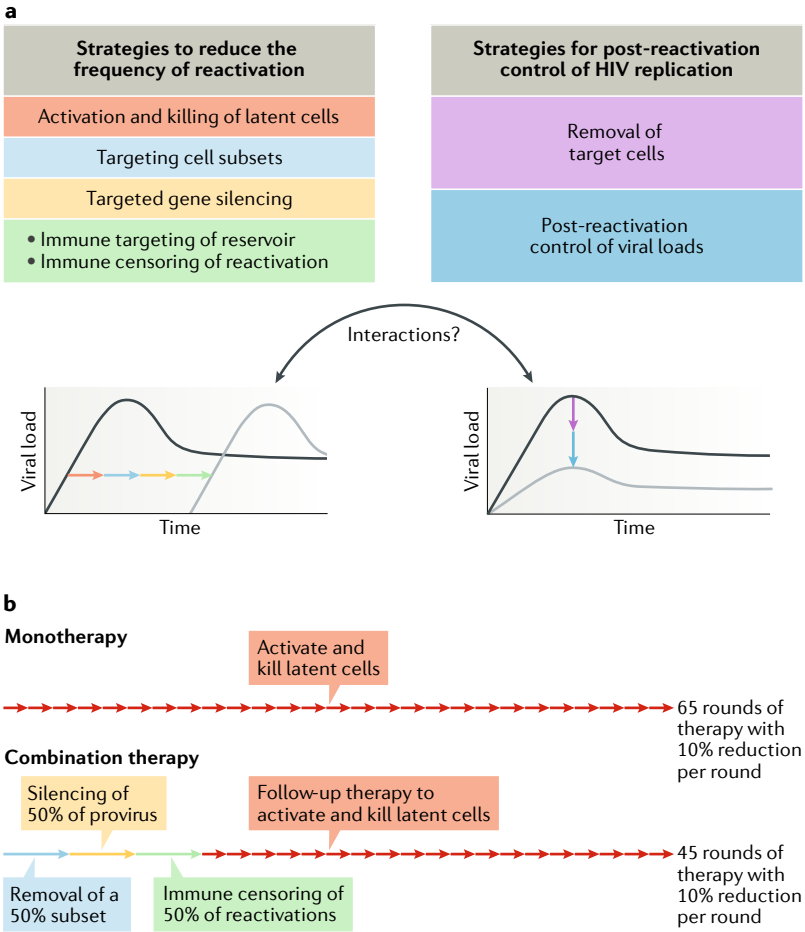


Fig. 5 | **Predicting synergies from combination therapy. a** | The approaches to inducing a functional cure of HIV can be divided into two categories: those aimed at reducing the frequency of reactivation from latency (delaying or preventing viral rebound) and those aimed at control of viral replication after reactivation has occurred. It is not clear whether these two actions would act synergistically. **b** | Combining strategies may act to reduce the overall efficacy needed from any individual treatment. For example, a therapy that reduced the frequency of reactivation by 10% per cycle would have to be given 65 times to achieve a 20-year remission. A combination therapy might include a treatment that removed a subset of latently infected cells to reduce reactivation by 50%, gene targeting to silence 50% of provirus and immune control blocking to reduce reactivation events by 50%. However, an additional 45 cycles of therapy that reduces the frequency of reactivation by 10% per cycle would still be required.

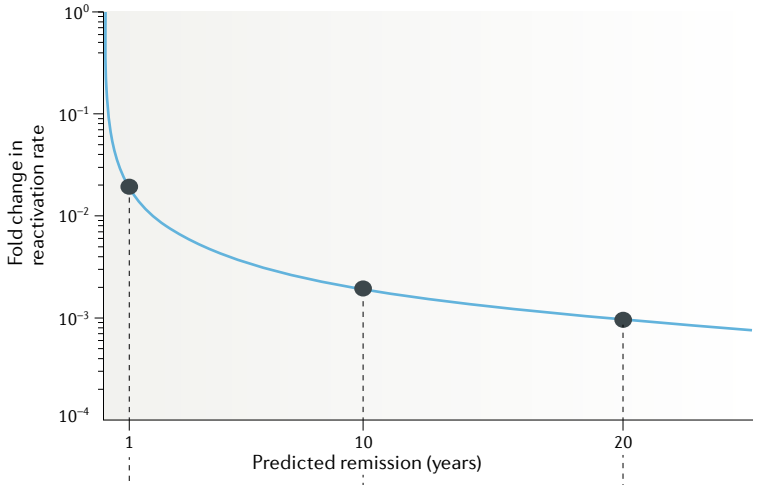
require high efficacy to contribute to the overall reduction required. For example, for approaches targeting a subset of highly infected cells, the ability to reduce the frequency of reactivation by 50% might be regarded as a considerable achievement. Similarly, if gene therapy could achieve gene silencing of HIV in 50% of latently infected cells, or if the immune response could block 50% of reactivation events, these would be major successes for these approaches. However, these would take us only a short way towards the level of reduction in reactivation that is needed. For example, FIG. 2 illustrates that an activation-and-death treatment that reduces the reactivation frequency by 10% per cycle would need to be given more than 65 times to achieve a 20-year remission. If the same activation-and-death treatment was included in a combination strategy involving a subset-targeting therapy that removes 50% of the reservoir, gene silencing that prevents HIV reactivation in 50% of the reservoir and a vaccine inducing immunity that blocked 50% of HIV reactivations, this combination therapy would reduce the number of treatment cycles needed from 65 to 45 cycles (FIG. 5b), unless major synergies can be achieved.

Strategies to reduce viral replication after initial viral rebound, such as inducing immune control or making target cells refractory to infection, would be expected to synergize to reduce post-reactivation viral loads (FIG. 5a, right panel). What is less clear is the extent to which synergy might occur between the frequency-reducing and post-reactivation-control strategies. For example, the Boston patients and Mississippi baby cases involved long delays between treatment interruption and viral reactivation from latency (indicating a greatly reduced frequency of reactivation), but high levels of viral replication occurred following recrudescence, suggesting that a low frequency of reactivation does not predict subsequent viral control[3,4]. This makes sense if we realize that frequency-reducing strategies might delay the time at which the first post-treatment viral reactivation event occurs (acting as the first infectious challenge to the host) (FIG. 1b), whereas strategies for post-reactivation viral control determine the trajectory of replication after viral growth has been initiated (FIG. 1c).

Conclusion

The potential long-term benefits of an HIV cure or durable remission have driven the development of several strategies to reduce the frequency of HIV reactivation from latency or to control the virus after reactivation. These are currently at an early stage and have yet to demonstrate notable post-treatment remission. However, a number of case studies of post-treatment control following early treatment or transplantation provide encouragement that remission is possible, given the right circumstances. In prioritizing the development of different agents, it is useful to consider the magnitude of effect that will be required using these different approaches (FIG. 6). For example, therapies aimed at reducing reservoir size must have a combination of primary efficacy (upon the first round of administration), durability (over multiple rounds) and tolerability to have any potential to achieve meaningful remission. Similarly, strategies to target $CD4^+$ T cell subsets are unlikely to succeed unless the subset contains the majority of infected cells. Strategies to induce post-rebound control of viral loads, either via making cells refractory to infection (via gene therapy) or by inducing host immune control, appear attractive. However, the mechanisms and potential of either gene therapy to produce refractory cells or boosted host immune control remain largely theoretical, and further work is required to demonstrate feasibility in vivo.

This review is based on the modelling of our current understanding of HIV reactivation and the effects of different therapeutic approaches. The baseline frequency of HIV reactivation from latency (that is, without any anti-latency interventions) in our Analysis was assumed to be on average once a week on the basis of a detailed analysis and the modelling of multiple clinical studies of



Latency-reducing treatment	Constant efficacy	10% per cycle, 38 cycles	15% per cycle, 38 cycles	20% per cycle, 31 cycles
	Waning efficacy (5% per cycle)	18 cycles, initially 30%		16 cycles, initially 50%
Targeting subsets	% of reservoir in subset	98% reservoir in subset		
Gene therapy	Removing provirus	98% of target cells reached		99.9% of target cells reached
	Cells refractory to infection	(Prevents recrudescence if >88% are inhibited)		
Vaccine/ immuno-therapy	Efficacy (% reactivations blocked)	98% efficacy	99.8% efficacy	
	Reservoir reduction	20% per year x 18 years	50% per year x 9 years	
	Post-reactivation control	(does not delay viral recrudescence)		

Fig. 6 | **Predicting the potency of different latency interventions.** Different interventions may act to reduce reservoir size, reduce the frequency of reactivation or control the viral load after reactivation. This table estimates the average duration of remission we might expect from different interventions on the basis of their effect on the underlying frequency of reactivation.

patients undergoing treatment interruption[1] (see BOX 1). The estimate of the baseline frequency of reactivation we used is lower than the estimate used in some earlier modelling studies, which have assumed either continuous viral production or frequencies of reactivation as high as four times a day[53,54]. If the baseline frequency of reactivation was higher than once a week, then more effective anti-latency treatments or a greater number of treatment cycles would be required to produce the same length of remission. Further work is clearly required to better characterize the basic determinants of HIV recrudescence from latency and subsequent HIV replication, which will allow a refinement in predictions of the effects of different interventions.

The number and diversity of strategies being pursued to tackle HIV latency speak to the importance and potential of this approach. Clearly, the challenges are large in many cases, and the scale of effect needed seems beyond the reach of current interventions. Modelling of treatment efficacy, scheduling and combination therapy has been used to propose optimal treatment strategies in oncology and to maximize remission and cure[55,56]. Quantitative considerations of the scale of the problem and the strength of interventions required can provide a rational guide for the development of agents to enable post-treatment remission of HIV.

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