

Curing HIV by ‘Kick and Kill’: From Theory to Practice?

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1. Introduction

Although antiretroviral therapy (ART) suppresses viraemia in HIV infected individuals, it is not a cure, and a treatment-resistant reservoir of latently infected cells remains. If ART is stopped viraemia returns, often rapidly. Lifetime ART, if available, involves side effects, cost and stigma. This has led to a great interest in curing HIV with diverse experimental approaches being considered ranging from therapeutic vaccination to gene therapy to bone marrow transplants with defective CCR5 genes.

One approach for achieving HIV cure has been termed ‘kick and kill’ (or ‘shock and kill’). Here, the reservoir is stimulated to reverse latency, and the now targetable cells are killed. Many of the concepts behind latency reversal were first considered over 25 years ago [1, 2], including the role of chromatin acetylation and methylation. However, it has only been in the last few years that these ideas have been tested clinically. Attractive in principle, the concept of ‘kick and kill’ has not been proven. Here, we explore the rationale, ethics and history of this approach, evidence for its effectiveness to date, and whether it is still a viable strategy or should be abandoned.

2. A ‘Kick’ is not enough.

Following the recognition of a latent HIV reservoir in resting CD4 T cells, approaches were devised to ‘flush out’ these residual proviruses. Based on the assumption that HIV replication might induce cell death, it was presumed that activating latently-infected cells would lead to their destruction [3].

This prompted the first studies exploring IL-2 to activate T cells to both stimulate HIV production and enhance CD4 T cell recovery through increased cell division. Chun et al [4] showed a decrease in IUPM ('Infectious Units Per Million' cells - a technique to measure replication competent provirus) with IL-2 and ART. However, there was no change in HIV DNA levels, possibly impacted by a dominance of replication-incompetent proviral sequences [5, 6]. This was, nevertheless, early evidence that activating the HIV reservoir could be successful. However, subsequent studies with IL-2 showed no impact on viral rebound kinetics on stopping ART [7]. Later studies combined OKT3 (a mouse anti-CD3 monoclonal antibody) with IL-2 to enhance T cell activation [8]. While effective at activating T cells and inducing HIV expression, there was no effect on HIV DNA or IUPM [8] suggesting that stimulation alone would not suffice.

Critically, there were significant safety concerns with these latter studies. Safety remains a crucial factor in kick and kill studies. The majority of participants will be well on antiretroviral therapy, with few side effects and effectively normal life expectancies. The decision, therefore, to introduce an experimental agent with potential potent side effects cannot be taken lightly. In addition, there is an increasing feeling that a treatment interruption (TI) is the only meaningful end-point in a clinical trial aimed at achieving remission, and this also raises ethical questions, particularly in regards to risks of onward transmission in viraemic participants. Deciding on the level of risk that the HIV community (patients, clinicians and researchers) is willing to accept will be critical in moving the HIV cure agenda forward [9, 10]. Because of these concerns, subsequent studies incorporated less potent activation strategies focusing on cytokines including IL-10 [11], IFN- γ [12] and IL-7 [13], none of which reduced the reservoir (and some appeared to increase it [14]).

3. Introducing the ‘kill’

Initial ‘kill’ approaches involved boosting immunity using treatment interruption (TI). It was hypothesised that multiple interruptions might act as a form of ‘self-vaccination’ to boost T cell responses. However, this approach only served to demonstrate that natural host HIV-specific immunity was inadequate [15], and that other approaches would be required to effectively target newly-awakened latently-infected cells. The history of vaccines in HIV is chequered. There is no effective preventative vaccine and little evidence that therapeutic vaccination is effective at reducing the HIV reservoir in humans [16, 17], although there have been promising studies in monkeys [18, 19].

‘Kick and Kill’ suggests an alternative role for vaccination; one might discriminate between a vaccine-induced immune response targeting latently infected cells versus one targeting cells actively producing virus, i.e. the difference between asking the immune system to target the reservoir (‘cure’) vs cells actively producing virions. These could require very different mechanisms, and there is a case to be made for the term ‘reservoir’ vaccine, which may require different immune system components beyond CD8 T cells [20].

4. Moving forward in the era of effective ART

With increasingly effective ART, newer anti-latency drugs were sought to drive out HIV expression. These included histone deacetylase inhibitors (HDACi), histone methyltransferase inhibitors, DNA methyltransferase inhibitors, bromodomain inhibitors, PKC agonists, and PI3K/AKT inhibitors as well as – more recently - SMAC mimetics and agonists for TLR7 or TLR9 (reviewed elsewhere [21]).

HDACi have been most widely tested, and clinical trials are on-going. Early results from *in vivo* studies [22, 23] showed increased HIV RNA transcription but did not translate into a decrease in reservoir size. *Ex vivo*, HDACi-treated cells are not more susceptible to T cell killing, although if exposed to heterologous highly effective T cells from ‘elite controllers’ there was evidence of increased cytotoxicity [24], arguably consistent with increased viral antigen presentation following HDACi administration.

Enhancing CD8 T cell function was the primary focus of kill efforts, which have now expanded to including other components of cellular immunity, such as natural killer (NK) cells, particularly due to advances in engineering broadly neutralising antibodies (bNAbs). Infusion of bNAbs reduces viraemia, delays viral rebound after ART interruption and enhances infected cell clearance [25]. The proposed role of bNAbs in enhancing NK and T cell function via possible combinations of antibody-dependent cellular cytotoxicity (ADCC), immune complex formation and dendritic cell engagement (the ‘vaccinal effect’) as well as their potential to elicit further effective antibody responses, suggest that an optimised ‘kill’ will require multiple arms of the immune system [26, 27].

5. Clinical Trials and HIV Cure

Only a few clinical studies have tested ‘kick and kill’ *in vivo*, with most employing T cell vaccines with HDACi. In the BCNO2-Romi trial, 15 participants received MVA.HIVconsv vaccination [28] with the HDACi romidepsin. At the time of writing, the results were not published, but at CROI 2017 it was reported that of 11 participants selected to stop ART, four remained off therapy (restart criteria >2000 copies/ml) for 7, 12, 14, and 22 weeks [29].

Follow-up of these individuals will be fascinating, but as short periods of apparent remission can be seen after ART alone, the data are hard to interpret without placebo controls [30]. One study using a different T cell vaccine (Vacc4x with rhuGM-CSF) with romidepsin reported a reduction in both HIV DNA and IUPM, and was the first to demonstrate a potential impact on the reservoir, although again without controls [31]. The first randomised controlled trial using an HDACi with a T cell vaccine was the RIVER trial where patients received a ChAdV63.HIVconsv vaccine with MVA.HIVconsv boost followed by vorinostat [32]. Despite increased histone acetylation and improved T cell function, there was no evidence of a reduced HIV reservoir as measured by HIV DNA (total or integrated) or viral outgrowth [32]. RIVER raises the key question of whether it showed no effect due to the futility of ‘kick and kill’ as a concept, or if the chosen drugs and vaccines were not potent enough.

6. Moving forward

Why have we not seen convincing outcomes from ‘kick and kill’ studies? Several findings may provide insight. A large fraction of integrated HIV DNA is replication competent but not activated in a single stimulation round *ex vivo*, even with potent stimuli [5]. If all latency cannot be reversed under optimised *ex vivo* conditions, the likelihood of a safe clinical intervention achieving this is minimal. It may be that the ‘kick’ (and possibly the ‘kill’) need to be given on multiple occasions over many months or years. Current clinical trial designs do not test this and - if we are looking for the cumulative summation of multiple small impacts - may not be sufficiently powered for proof of principle. If the kick is effective and antigen is presented, the immune system might still not provide adequate ‘kill’ due to immune ‘distractions’ caused by replication incompetent viruses producing non cross-reactive viral proteins [33], due to persistent and non-reversible functional T cell exhaustion

[34], or to innate resistance of the reservoir to CTL[20]. One of the key barriers to effective immunity – the development of viral immune escape – should be less of an issue for a ‘reservoir’ vaccine, as the absence of circulating viraemia on ART should remove from the equation error-prone viral replication and the opportunity to adapt to immune-induced selection pressure. Nevertheless, even if CTL were effective at eliminating reactivated cells, whether these effectors are able to effectively reach the necessary anatomical sites (eg germinal centres) in order to reduce the HIV reservoir remains unclear [35]. Thus, if it exists, the right ‘kill’ is likely to be in the form of a combination of multiple immune enhancing approaches, including possible engineered agents such as CAR T cells (reviewed in [21, 25]).

7. Conclusions

Progress in achieving HIV remission through ‘kick and kill’ has been limited. Whether this is due to the futility of the concept or the ineffectiveness of currently available agents remains to be seen. However, the premise remains theoretically attractive, and it remains the most scalable approach to reducing the HIV reservoir.

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