



ARTIST  
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**CRITICAL REVIEW**

# Understanding Shock and Kill

A POTENTIAL HIV CURE

The proviral latency of human immunodeficiency virus (HIV) remains a principal obstacle to curing the infection and halting disease pathogenesis. In fact, HIV infection remains incurable due to a long-lived latent reservoir of HIV within the central nervous system (CNS), as well as the most impervious reservoir, CD4<sup>+</sup> T cells. This review evaluates “shock and kill” therapy, an emerging curative strategy that targets HIV reservoirs. The novel approach aims to “shock” latently infected cells by inducing HIV viral expression through latency reversing agents (LRA). Upon reactivation, the “kill” method refers to the termination of virus-infected cells by employing strategies involving HIV-specific CD8<sup>+</sup> T cells. The potential benefits of purging immune cells of HIV-infected individuals and limiting the size of the latent reservoir must be assessed to advance the “shock and kill” technique.

## HIV PATHOGENESIS

HIV is a retrovirus whose hallmark replicative strategy employs reverse transcription to convert viral RNA into double-stranded DNA (dsDNA). This process generates a significant number of mutations in HIV, since reverse transcriptase is an error-prone enzyme that lacks a proofreading mechanism. Subsequent integration of the viral DNA into the host cell genome allows HIV to successfully utilize cell machinery to synthesize viral components.<sup>1</sup> Assembled viral proteins are then exported via exploitation of the vesicular sorting pathway.<sup>2</sup> Accelerated cell destruction and viral proliferation are multifactorial, as virions hijack apoptotic signalling, induce direct cytotoxicity, and initiate atypical immune activation.<sup>3</sup>

Notably, HIV can infect both dividing and nondividing cells, such as neuronal glial cells, macrophages, and CD4<sup>+</sup> T cells, with CD4<sup>+</sup> T cells undergoing the highest rates of HIV infection.<sup>1</sup> Hence, the virus is present in a myriad of organs, including the brain, gut, and placenta. Considered to be a part of the hematopoietic system, CD4<sup>+</sup> T cells are immune cells that play a critical role in facilitating adaptive immunity.<sup>4</sup> In response to an antigen, resting CD4<sup>+</sup> T cells activate, proliferate, and differentiate to generate short-

lived effector CD4<sup>+</sup> T cells, which activate the adaptive immune system to defend the body.<sup>5</sup> Most effector CD4<sup>+</sup> T cells terminate, but a portion persists as memory cells that contribute to altered gene expression and enable long-term survival. Following integration into the host cell genome, HIV DNA is transcriptionally silent and unaffected by antiretroviral (ARV) medications, giving rise to the phenomenon of viral latency. Ultimately, HIV latency exploits the immune system’s most intrinsic feature: the immunological memory that dwells within T cells.<sup>6</sup>

Antiretroviral therapy (ART) employs a combination of ARV drugs that suppress HIV and halt disease progression by inhibiting viral replication.<sup>7</sup> Treatment of HIV infection with a combination of three to five ARV drugs is more efficacious than

monotherapy, and greatly reduces the odds of drug resistance. However, complete eradication of HIV in patients undergoing ART cannot be achieved due to the presence of the latent HIV reservoir.<sup>5</sup> Moreover, ART is a lifelong treatment that requires rigorous adherence to maintain therapeutic drug levels. However, prolonged treatment is often unattainable in poor socioeconomic regions with low patient compliance, thus contributing to increased drug resistance and a decreased quality of life.<sup>8</sup>

Multiple classes of drugs are administered in ART to target specific stages of HIV pathogenesis; the earliest include nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors.<sup>7</sup> Recently, additional classes such as fusion and integrase inhibitors have emerged. Despite its therapeutic benefits, multidrug ART is accompanied by adverse side effects which can lead to chronic illnesses.<sup>2</sup> Such conditions include liver toxicity and hypersensitivity syndrome, the latter of which develops due to a rigorous immune response to NRTIs and fusion inhibitors. The profound burdens of the growing HIV epidemic and associated chronic diseases on global health and primary care highlight the need to further investigate existing treatment methods.<sup>10</sup>

## OVERVIEW OF THERAPEUTIC MECHANISM

The pressing need for an HIV cure has spurred a novel therapeutic approach that aims to eradicate the virus by depleting HIV reservoirs. Commonly referred to as “shock and kill”, this therapy entails shocking latently infected cells by inducing HIV viral expression and killing them following reactivation. A drug which reverses latency increases HIV RNA levels to induce viral protein production.<sup>11</sup> Following the shock stage, the viral particles are released from the infected cells to be later purged by the immune system.<sup>12</sup> Recent advances in HIV research have highlighted shock and kill as a promising cure. In fact, multiple classes of latency reversing agents (LRAs) have already been identified, with a select few currently undergoing clinical investigation. Moreover, recent studies have identified a novel method that achieves viral eradication by exploiting HIV-specific CD8<sup>+</sup> T cells, a subset of immune cells that can recognize and kill infected cells.<sup>11</sup> To prevent future infections, ART treatment must be maintained during shock and kill.<sup>12</sup>

### THE “SHOCK” ASPECT

Different pathways can be targeted to increase HIV cellular transcription. One promising approach involves the upregulation of gene expression via epigenetic means. Existing enzymes, such as histone deacetylase and histone methyltransferase, maintain HIV latency by reducing DNA accessibility to transcription factors and inducing histone trimethylation to repress genetic expression, respectively.<sup>13</sup> Consequently, histone deacetylase inhibitors and histone methyltransferase inhibitors have been subjects of rigorous investigation.<sup>13,14</sup> A recent study of eight HIV patients demonstrated that treatment with vorinostat, a histone deacetylase inhibitor, caused a 4.8 fold increase in HIV RNA expression just six hours post-treatment.<sup>15</sup> Histone methyltransferase inhibitors such as chaetocin and BIX-01294 have also been effective as LRAs in *in vitro* and *ex vivo* studies. In addition to epigenetic mechanisms, induction of positive transcription factors can further increase HIV viral expression to expose latently infected cells.<sup>13</sup>

Inducible transcription factors, including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), are naturally present at very low levels in resting cells and are located outside of the nucleus, away from

transcriptional machinery. The inhibitor of NF-κB (IκB) is responsible for the sequestration of this transcription factor in the cytoplasm. Consequently, inhibition of IκB allows NF-κB to enter the nucleus, where it can activate HIV promoter region.<sup>13</sup> Positive transcription elongation factor (PTEF-b) is another essential protein that plays a critical role in the maintenance of HIV latency. The viral protein trans-activator of transcription (TAT) is responsible for recruiting PTEF-b during the elongation phase of transcription. During this process, TAT must directly compete for PTEF-b with another protein, bromodomain-containing protein 4 (BRD4). As a result, inhibitors of BRD4 such as JQ1 have been shown to increase viral transcription in cell line models.<sup>15</sup>

Perhaps the most effective strategy to reverse latency entails the activation of phosphokinase C (PKC). Stimulating the PKC pathway improves HIV transcription through two related mechanisms: the release of positive transcription factor NF-κB inside the nucleus, and the induction of PTEF-b production and availability.<sup>13</sup>

### THE “KILL” ASPECT

After reactivation, the kill stage of therapy entails the purging of infected cells to eradicate viral reservoir and cure patients. It has been shown that HIV-specific CD8<sup>+</sup> T cells can effectively kill reactivated CD4<sup>+</sup> T cells *in vitro*.<sup>16</sup> Multiple strategies have utilized HIV-specific CD8<sup>+</sup> T cells to achieve viral eradication. Among these, adaptive transfer of HIV-specific CD8<sup>+</sup> T cells, therapeutic vaccines, and immunomodulation have been intensely studied.<sup>11</sup>

Although HIV-infected individuals possess HIV-specific CD8<sup>+</sup> T cells which are initially capable of killing infected cells, their effectiveness is gradually lost. In fact, HIV-specific CD8<sup>+</sup> T cells isolated from acute and chronic HIV infection show functional and phenotypic differences. Therefore, the adaptive transfer of HIV-specific CD8<sup>+</sup> T cells, which are derived *in vitro* to have increased persistence, may be a safe and effective approach to purging the infected cells.<sup>17</sup> Therapeutic vaccines created using live attenuated vectors, peptides, and proteins have shown promising results in inducing CD8<sup>+</sup> T cells. Boosting CD8<sup>+</sup> T cells can also be achieved using immunomodulators that have been experimentally effective in reversing CD8<sup>+</sup> T cell exhaustion.<sup>11</sup>

## KEY CONSIDERATIONS

Despite the scientific promise of the shock and kill technique, one must consider the therapy's efficacy as well as associated side effects. Notably, the approach relies on the condition that all latently infected cells will be re-activated and subsequently eliminated by cytotoxic T lymphocytes, an assumption that lacks sufficient evidence. It is speculated that the usage of weak LRAs accounts for this inadequacy, highlighting the need for more efficacious agents. Moreover, ART cannot be presumed to completely block the released virions from newly infected cells. Specifically, cells localized in the brain and lymphoid tissues may not receive the threshold concentration of ARV drugs required for viral suppression. This renders the efficacy of drugs that target reactivated viruses in these anatomical sites highly debatable. Another potential ramification associated with LRAs is that they can act as agonists which activate uninfected T cells, rendering these cells vulnerable to HIV infection. Other non-HIV viruses, including human herpes and hepatitis C viruses, can similarly be reactivated, leading to detrimental side effects. Further LRA-associated conditions may result from the targeting of host factors and the modulation of host gene expression, which include inheritable epigenetic markers.<sup>18</sup>

The shock and kill technique should be closely monitored, since HIV is associated with chronic diseases such as various neurocognitive disorders. Studies have suggested that reversed latency increases the risk of acute HIV syndrome-associated encephalitis, otherwise known as

brain inflammation.<sup>19</sup> Several LRAs induce dose-dependent breakdown of endothelial integrity and permeabilize the blood-brain barrier (BBB). Although this could improve the access of ART drugs to the CNS during shock and kill, opening of the BBB is often associated with neurological diseases. Moreover, LRAs such as prostratin and bryostatin-1 cause the microvascular endothelial cells of the brain to secrete inflammatory factors, a phenomenon associated with disease progression. Evidently, more research is required to determine the safety of these drugs.<sup>20</sup>

## CONCLUSION

Current HIV treatments are limited as they can only halt disease progression and require strict adherence to a rigorous drug regimen. With the advent of the shock and kill approach, the research community has become increasingly optimistic towards a potential cure for HIV. This technique is novel in that it ultimately targets the very machinery by which the virus evades the immune system and resides within host cells. If efficacious, the therapy can curb the current global HIV pandemic and simultaneously increase the quality of life of patients inflicted with the disease. However, it is pertinent to remain cognizant of the ramifications associated with shock and kill. The reversal of latency can trigger potentially detrimental side effects for patients, which is a high-priority area of research that requires thorough exploration. ■

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