

# Toll-Like Receptor Agonists: Can They Exact a Toll on Human Immunodeficiency Virus Persistence?

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(See the Major Article by Vibholm et al on pages 1686–95.)

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In this issue of *Clinical Infectious Diseases*, Vibholm *et al* report on the first clinical trial of treatment with MGN1703 (leftolimod), an agonist of Toll-like receptor 9 (TLR9), as a potential strategy toward achieving a “functional cure” for human immunodeficiency virus (HIV) infection, defined as prolonged control of viremia without antiretroviral therapy (ART). After reading the report, two questions are likely to come to mind: What are Toll-like receptors, and how might their stimulation affect HIV that persists within the infected individual and leads to relapse despite prolonged, clinically effective ART?

Toll-like receptors are a subfamily of pattern recognition receptors that recognize various unique components of prokaryotic, fungal, and viral pathogens and that stimulate key innate and adaptive immune responses to infection. They are named TLR because of their structural and functional similarity to the *Drosophila* transmembrane protein, Toll [1, 2]. TLR agonists, like MGN1703, mimic pathogen-associated molecular patterns (PAMPs) and can specifically

enhance innate and adaptive responses. Unlike most of the TLR family, TLR3, TLR7, TLR8, and TLR9 are not located on the cell surface but rather on endosomal membranes—permitting the sensing of nucleic acid from intracellular pathogens. TLR3 is expressed primarily within dendritic cells (DCs) and senses retroviral double-stranded RNA (dsRNA), leading to the activation of interferon regulatory factor (IRF3), independent of the signaling adapter MyD88 [3, 4]. TLR7 and TLR8 sense single-stranded RNA and signal through the adapter protein MyD88 to activate interferon regulatory factors IRF5 and IRF7, leading to the activation of NF- $\kappa$ B and production of type I interferons (IFNs). TLR7 is expressed exclusively within plasmacytoid dendritic cells (pDCs) and B cells, whereas TLR8 is expressed within myeloid DCs [5–8]. TLR9, the target of MGN1703, is similar to the other intracellular TLRs but recognizes CpG-rich hypomethylated DNA motifs and signals through MyD88-dependent mechanisms to activate IRF7 in pDCs [9–11]. Although binding of PAMPs to intracellular TLR triggers a variety of signaling mechanisms, they all converge on the transcription and production of type I IFNs from antigen-presenting cells, which have a critical role in protective immunity [12, 13].

Clinical applications of TLR agonists are common; they are used as antitumor agents for various types of cancer

and as vaccine adjuvants. Bacterial BCG is a US Food and Drug Administration (FDA)-approved TLR1–2 agonist. Monophosphoryl lipid A, a TLR4 agonist, is an effective and nontoxic adjuvant vaccine adjuvant [14–16], as is polyinosinic:polycytidylic acid, a TLR3 agonist [17, 18]. The imidazoquinolinamines resiquimod and imiquimod are TLR7/8 agonists, which induce innate and adaptive immune responses, and imiquimod is currently FDA approved as a topical treatment for genital warts and superficial basal cell carcinoma [19–23].

Why, then, is TLR agonism being pursued as a means of eliminating or controlling HIV persistence? As broad stimulants of innate and adaptive immune responses, TLR agonism may affect HIV reservoirs through the widely pursued “kick and kill” approach to HIV cure [24]. This “kick and kill” approach aims to eliminate HIV-infected cells in 2 ways: (1) by reversing HIV latency, a major mechanism whereby HIV remains invisible to the immune system, and (2) by killing the newly exposed, HIV-infected cells through either direct viral protein-induced cytopathic effect or through immune-mediated mechanisms such as major histocompatibility complex (MHC) class I-restricted HIV-specific cytotoxic CD8<sup>+</sup> T cells, cytotoxic natural killer (NK) cells, or antibody-dependent cytotoxic functions.

Regarding the “kick” component of “kick and kill,” the latency reversing

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agents discovered to date reactivate only a very small proportion of latent proviruses *ex vivo* [25, 26]. *In vivo*, histone deacetylase inhibitors (vorinostat, panobinostat, and romidepsin) have been studied most widely as latency reversing agents, having modest effects on proviral transcription and variable effects on persistent viremia, but none has reduced the latent HIV reservoir [27–29]. By contrast, initial studies with the TLR7 agonists GS-986 (preclinical compound only) and GS-9620 (vesatolimod; in clinical development) have shown more promise in achieving both “kick and kill.” In nonhuman primates on suppressive ART, repeated dosing with GS-986 and GS-9620 consistently induced plasma HIV RNA blips, activated interferon-stimulated genes (ISGs) and multiple cell types of the immune system, reduced simian immunodeficiency virus (SIV) proviral DNA, and cleared all evidence of residual SIV infection in 2 of 11 monkeys studied [30]. These promising preclinical results are being further evaluated in an ongoing human clinical trial of GS-9620, although no data are yet available.

The pioneering human study of MGN1703 reported by Vibholm *et al* sought to reverse HIV latency and enhance killing of HIV-infected cells by administering twice-weekly subcutaneous doses of 60 mg for 4 weeks to 15 HIV-infected, ART-suppressed study participants. Existing TLR9 agonists are CpG oligonucleotides that have been used in the treatment of non-Hodgkin lymphoma [31, 32], metastatic melanoma [33, 34], and as a vaccine adjuvant [35]. The compound used in this study (MGN1703) is in phase 3 testing for treatment of metastatic colorectal cancer but differs from standard CpG motifs in that its structure comprises 2 loops of 30 nucleotides containing 3 CG motifs each connected by a stem of 28 base pairs, creating a unique “dumbbell” shape [36].

In the current trial, changes in NK cell activation after weeks of dosing was the primary study endpoint. The treatment appeared to be generally well tolerated

although transient neutropenia (grade 1–3) deserves follow-up in future studies. The authors report significant increases in the expression of activation markers CD86 and CD40 on pDCs and CD69 on NK cells, thus meeting the primary study endpoint. Levels of HLA-DR/CD38 expression on effector and central memory CD4<sup>+</sup> and CD8<sup>+</sup> T cells were modestly increased, while plasma cytokine levels of IFN- $\alpha$ 2, IFN- $\gamma$ , TNF, and CXCL10 were significantly elevated, as were transcription of the ISGs OAS1, MX1, and ISG15. Taken together, these findings show clear evidence of immunologic responses after multiple doses of MGN1703, with significant activation of NK cells, pDCs, and increases in IFN-related genes and cytokines. Whether HIV-specific immune functions were increased was not reported and is paramount for evaluating progress toward immune control of HIV. The effects of MGN1703 treatment on virologic parameters are more subtle. Without a control arm or an extended period of observation before MGN1703 dosing, it is difficult to interpret the changes in viremia or cell-associated HIV RNA that were observed. The authors acknowledge this limitation of their initial pilot study. Six of 15 individuals showed isolated plasma HIV RNA blips at some time during the 4-week dosing period. One individual had an impressive plasma HIV RNA spike to >1000 copies/mL, but without antiretroviral drug levels it is difficult to differentiate latency reversal induced by MGN1703 from temporary nonadherence to ART.

Does this study bring the field closer to a functional HIV cure? The work is an initial, small step forward, showing that TLR9 agonism has immunologic effects in HIV-infected individuals that are not different from what was expected or observed in other patient groups, such as those with cancer, but that short-term TLR9 agonism has no lasting impact on the HIV reservoir. Nevertheless, the approach seems to be safe and the results are likely to inform the design of future

studies. It may be that the dosing schedule in this study was too frequent (twice weekly) and that longer-term, less-frequent dosing may be required to impact HIV reservoirs. Combining TLR9 agonism with therapeutic vaccination to enhance immune responses and direct them toward conserved viral epitopes is another alternative, which has recently shown promise in nonhuman primates with the TLR7 agonist GS-986 [37].

There are many divergent approaches being taken to achieve a functional HIV cure, ranging from monoclonal antibodies designed to broadly cover HIV variants and enhance Fc-mediated effector functions, to gene modification of host T cells to block susceptibility to HIV or to generate potent effector cells with chimeric antigen receptors directed against HIV, as has been achieved with remarkable success for CD19 in B cell malignancies [38–41]. As was the case for the evolution of highly effective ART, finding the right combinations of TLR agonists, therapeutic vaccines, and broadly neutralizing monoclonal antibodies through iterative evaluations will be necessary to achieve a functional cure of HIV for all patients. Although TLR agonism shows considerable promise for exposing latent HIV-infected cells and for enhancing their clearance, much work needs to be done before the toll that such agonists can take on HIV persistence is defined.

## Note

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