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Commentary

Preventive RNAi-based Microbicide to Inhibit HIV Transmission in the Vaginal Mucosa

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Commentary

With annually ~1.5 million new HIV infections worldwide, the HIV/AIDS pandemic remains a significant global health burden [1]. While antiretroviral therapy is highly effective to manage and slow down disease progression, a preventive effective vaccine to control the pandemic remains elusive [2]. Currently available preventive strategies such as the use of condoms, and HIV pre- and post-exposure prophylaxis are effective, but the lack of compliance remains challenging [3-5]. To end the HIV/AIDS pandemic, these existing treatment and prevention modalities need to be combined with an effective preventive vaccine, which is not available yet [5].

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Given the difficulties in developing an HIV vaccine, a vaginal microbicide based on RNA interference (RNAi) technology might be an attractive approach to eradicate the pandemic by inhibiting heterosexual HIV transmission, which accounts for most new HIV infections worldwide [6,7].

Upon its discovery in 1998, RNAi quickly emerged as powerful tool to silence gene expression, which was applied to prevent and treat disease, and study the function of genes. In 2018, the FDA and EMA approved the first RNAi-based drug named patisiran, which opened the door for a novel class of RNAi-based therapeutics. Currently, six siRNA-based therapeutics are commercially available to treat various conditions [8].

Our approach was to develop a preventive vaginal HIV microbicide based on the topical application of RNAi technology. By harnessing cholesterol-conjugated small interfering RNAs (chol-siRNAs) as mediators of RNAi-based gene silencing, our goal was to modulate gene expression in murine mucosal immune cells *in vivo* as a model for inhibiting heterosexual HIV transmission [9].

Our proof-of-concept study was based on previous findings in our lab, which showed that intravenous application of siRNAs in mice was functional *in vivo*. Injection of Fas-targeting siRNAs silenced Fas expression in murine hepatocytes *in vivo*. Upon challenge of mice with concanavalin A, intravenous administration of Fas-targeting siRNAs prevented hepatic fibrosis, thus manifesting the therapeutic potential of RNAi *in vivo* [10].

Another proof-of-principle study showed the successful topical application of siRNAs in the murine Cervicovaginal Tract (CVT) *in vivo*, as Intravaginally (IVAG) instilled lipoplexed siRNAs targeting two HSV-2 host genes protected mice from a lethal challenge with HSV-2 [11]. In a subsequent study our colleagues Yichao Wu et al demonstrated that IVAG application of cholesterol-conjugated siRNAs inhibited HSV-2 infection by silencing expression of Nectin-1, which is a host protein mediating HSV-2 entry in the epithelial layer of the CVT. Co-targeting an antiviral and cellular gene such as Nectin-1 proved to be more effective than knockdown of Nectin-1 alone [12].

In contrast to lipoplexed siRNAs, chol-siRNAs didn`t trigger an interferon response or cause any detectable inflammation [12]. Since inflammation induced by sexually transmitted diseases is an important factor that enhances mucosal susceptibility to HIV infection, chol-siRNAs are likely to be more suitable as component of an HIV microbicide [13,14].

Based on our previous studies, we embarked towards harnessing topically applied chol-siRNAs to modulate gene expression in murine mucosal immune cells *in vivo* [9]. Our proof-of-concept study`s goal was to demonstrate that chol-siRNAs can be used to target various immune cell populations within the murine CVT and silence host genes that are essential for HIV infection and viral dissemination.

We chose CD45 as a target gene to suppress, as it is widely and uniformly expressed on human and murine immune cells such as dendritic cells, macrophages and T lymphocytes. To evaluate cellular uptake and functionality of siRNAs *in vivo*, we treated mice intravaginally with siRNAs, extracted immune cells from vaginal tissue, and

used a migration assay to isolate viable immune cells, which were analysed by multichannel flow cytometry [9].

Upon intravaginal instillation of Cy3-labeled chol-siRNAs, we found that migrated cells were predominantly $CD11b^+$ $CD45^+$. Flow cytometry analysis showed that they efficiently incorporated chol-siRNAs. However, when treating mice with chol-siRNAs targeting CD45, gating on $CD11^+$ $F4/80^+$ macrophages revealed that CD45 knockdown was minimal (around 20%) compared to mock-treated mice and thus unlikely to translate into a therapeutic effect [9].

It turned out to be extremely challenging to isolate different viable mucosal immune cell populations from collagenase-digested vaginal tissue for flow cytometry analysis. Therefore, we developed a new computational imaging approach based on confocal fluorescence microscopy to quantify gene silencing of mucosal immune cells *in situ* upon intravaginal administration of chol-siRNAs into mice *in vivo*.

First, we showed that intravaginally applied Cy3-labeled cholesterol-conjugated siRNAs penetrate the entire vaginal mucosa of mice including the lamina propria (LP).

In a second step, we harnessed our quantitative imaging method to measure gene knockdown of immune cells *in situ*. Compared to mock-treated mice, intravaginal instillation of CD45 chol-siRNAs resulted in (partial) gene silencing in intraepithelial (IE) and lamina propria (LP) dendritic cells. However, we could not detect any effective gene silencing in $CD11b^+$ $CD45^+$ cells and $CD3\varepsilon^+$ $CD45^+$ T cells *in situ* [9].

Taken together, our research demonstrated penetration of intravaginally administered chol-siRNAs into the vaginal mucosa including the LP *in vivo*. Chol-siRNAs were efficiently taken up by intraepithelial and lamina propria DCs, as well as $CD11b^+$ $CD45^+$ cells, but not by T cells. While chol-siRNAs induced partial gene silencing in IE and LP DCs throughout the vaginal mucosa *in vivo*, they were ineffective in $F4/80^+$ $CD11b^+$ macrophages and $CD3\varepsilon^+$ $CD45^+$ T cells.

Despite several decades of research, the exact mechanisms underlying HIV infection remain poorly understood [15]. New evidence suggests that mucosal entry of HIV and its subsequent propagation not only involve multiple immune cell populations but also epithelial cells (ECs) [15-17]. ECs and mucosal DCs are considered as first targets of HIV, mediating viral binding/uptake, sequestration, and transfer to HIV-susceptible cells [15-20].

Mucosal DCs play a particularly important role in HIV infection: as sentinel cells they capture virions using their C-type lectin receptors. Upon binding of virions, they upregulate the chemokine receptor CCR7, allowing them to migrate from the cervicovaginal mucosa to afferent lymphoid tissues where they transmit captured virus to HIV-susceptible cells. Multiple studies demonstrated the critical role of actin control genes in DCs to allow trans-infection [15,18-21].

Suppression of DC proteins that regulate binding of HIV-1 (CD169/Siglec-1, DC-SIGN), cellular maturation and migration (CCR7), and actin organization (TSPAN7, DNM2) might be impactful to inhibit DC-mediated HIV uptake, its transportation to lymph nodes and trans-infection of $CD4^+$ T cells.

Consequently, the most promising approach to disrupt HIV infection and dissemination might be to use multiplexes of siRNAs for targeting a spectrum of critical host genes in both ECs and mucosal DCs, thereby inhibiting HIV uptake, viral sequestration, cell migration and cell-to-cell spread of HIV-1 to $CD4^+$ T lymphocytes.

Our study and the *in vivo* application of RNAi as a therapeutic agent may have several limitations.

Successful siRNA-mediated gene silencing in mice does not automatically translate into its effectiveness in humans. However, the successful application of CD4 aptamer-siRNA chimeras to inhibit HIV transmission in humanized mice and the approval of multiple siRNA-based therapeutics for humans suggest siRNAs to be a promising compound for a topical microbicide that could be effective in humans [8,22].

The stability of chemically unmodified siRNAs is endangered by endogenous RNAses in the vaginal tract [12]. Several advancements in siRNA synthesis (Phosphorothioate backbone modifications, integration of phosphorothioate residues) as well as siRNA conjugation and packaging (liposomes) dramatically improved siRNA stability and durability *in vivo* [23].

Furthermore, chemical modifications of siRNAs including conjugation to cholesterol have been shown to optimize delivery and endolysosomal escape upon cellular uptake, which is required to allow siRNAs to reach the P-bodies where the silencing machinery is located [24].

Design enhancements including sequence optimization and chemical modifications, and careful selection of target genes may help reduce off-target effects, which are a typical drawback of applying RNAi [7].

The targeted delivery of siRNAs remains a major challenge for the therapeutic application of RNAi. The *in vivo* transfection of immune cells is particularly difficult [22,25]. However, we showed that topical application of chol-siRNAs could modulate gene expression in epithelial cells and mucosal dendritic cells simultaneously within the murine CVT [9,12]. Targeting these two cell types might not be sufficient to completely inhibit HIV infection though. Addition of newly designed targeted siRNAs might help expand the reach to other cells that are critical for HIV infection such as T cells, macrophages and fibroblasts [25].

In conclusion, our study suggests that chol-siRNAs might serve as an elegant tool to study the role of mucosal dendritic cells in HIV transmission and viral dissemination.

The lack of an effective HIV vaccine necessitates alternative preventive approaches. Research by us and others showed topically administered chol-siRNAs to be effective in modulating gene expression in several mucosal cell populations which are critical for HIV infection. These findings are a first promising step towards a preventive HIV microbicide. Ideally, a vaginal microbicide would contain a cocktail of stabilized siRNAs targeting multiple host and viral genes in all HIV relevant cell populations, aiming at minimizing HIV entry and viral sequestration, limiting DC migration and inhibiting DC-mediated *trans*-infection of T cells, and hence preventing subsequent viral dissemination.

Conflict of Interest Disclosure

EB none, ASK none, BS none, IR none, AJ none, NHB none

References

1. Haynes BF, Wiehe K, Borrow P, Saunders KO, Korber B, et al. (2023) Strategies for HIV-1 vaccines that induce broadly neutralizing antibodies. Nat Rev Immunol 23: 142-158.
2. Fortner A, Bucur O (2022) mRNA-based vaccine technology for HIV. Discov Craiova Rom 10: 150.
3. Ahaus P, Schmidt AJ, Skaletz-Rorowski A, Uhrmacher M, Serova K, et al. (2022) Changes in the user profiles of HIV pre-exposure prophylaxis (PrEP) before and after PrEP reimbursement. J Infect Public Health. 15: 955-960.
4. Agrahari V, Anderson SM, Peet MM, Wong AP, Singh ON, et al. (2022) Long-acting HIV pre-exposure prophylaxis (PrEP) approaches: recent advances, emerging technologies, and development challenges. Expert Opin Drug Deliv 19: 1365-1380.
5. Eisinger RW, Fauci AS (2018) Ending the HIV/AIDS Pandemic(1). Emerg Infect Dis 24: 413-416.
6. Blondin-Ladrie L, Aranguren M, Doyon-Laliberté K, Poudrier J, Roger M (2021) The Importance of Regulation in Natural Immunity to HIV. Vaccines 9.
7. Wheeler LA (2014) Silencing sexually transmitted infections: topical siRNA-based interventions for the prevention of HIV and HSV. Infect Dis Obstet Gynecol 2014: 125087.
8. Guo S, Zhang M, Huang Y (2024) Three “E” challenges for siRNA drug development. Trends Mol Med. 30: 13-24.
9. Basar E, Shum B, Skaletz-Rorowski A, Wu Y, Nambiar S, et al. (2023) Cholesterol-conjugated siRNAs silence gene expression in mucosal dendritic cells in cervicovaginal tissue in mice. J Eur Acad Dermatol Venereol. 37: 615-626.
10. Song E, Lee SK, Wang J, Ince N, Ouyang N, et al. (2003) RNA interference targeting Fas protects mice from fulminant hepatitis. Nat Med 9: 347-351.
11. Palliser D, Chowdhury D, Wang QY, Lee SJ, Bronson RT, et al. (2006) An siRNA-based microbicide protects mice from lethal herpes simplex virus 2 infection. Nature 439: 89-94.
12. Wu Y, Navarro F, Lal A, Basar E, Pandey RK, et al. (2009) Durable protection from Herpes Simplex Virus-2 transmission following intravaginal application of siRNAs targeting both a viral and host gene. Cell Host Microbe 5: 84-94.
13. Mwatelah R, McKinnon LR, Baxter C, Abdool Karim Q, Abdool Karim SS (2019) Mechanisms of sexually transmitted infection-induced inflammation in women: implications for HIV risk. J Int AIDS Soc 6: 25346.
14. Mtshali A, Ngcapu S, Mindel A, Garrett N, Liebenberg L (2021) HIV susceptibility in women: The roles of genital inflammation, sexually transmitted infections and the genital microbiome. J Reprod Immunol 145: 103291.
15. Hladik F, McElrath MJ (2008) Setting the stage: host invasion by HIV. Nat Rev Immunol 8: 447-457.9
16. Wu Z, Chen Z, Phillips DM (2003) Human genital epithelial cells capture cell-free human immunodeficiency virus type 1 and transmit the virus to CD4+ Cells: implications for mechanisms of sexual transmission. J Infect Dis 188: 1473-1482.
17. Yasen A, Herrera R, Rosbe K, Lien K, Tugizov SM (2018) HIV internalization into oral and genital epithelial cells by endocytosis and macropinocytosis leads to viral sequestration in the vesicles. Virology 515: 92-107.
18. Sewald X, Ladinsky MS, Uchil PD, Beloor J, Pi R, et al. (2015) Retroviruses use CD169-mediated trans-infection of permissive lymphocytes to establish infection. Science 350: 563-567.
19. Matsuzawa T, Ogawa Y, Moriishi K, Shimada S, Kawamura T (2017) Immunological function of Langerhans cells in HIV infection. J Dermatol Sci 87: 159-167.
20. Gummuluru S, Pina Ramirez NG, Akiyama H (2014) CD169-dependent cell-associated HIV-1 transmission: a driver of virus dissemination. J Infect Dis 3: 641-647.
21. Donahue DA, Schwartz O (2016) Actin’ on HIV: How Dendritic Cells Spread Infection. Cell Host Microbe 19: 267-269.
22. Wheeler LA, Trifonova R, Vrbanac V, Basar E, McKernan S, et al. (2011) Inhibition of HIV transmission in human cervicovaginal explants and humanized mice using CD4 aptamer-siRNA chimeras. J Clin Invest 121: 2401-2412.
23. Setten RL, Rossi JJ, Si-Ping H (2019) The current state and future directions of RNAi-based therapeutics. Nat Rev Drug Discov 18: 421-446.
24. Dykxhoorn DM (2005) P-bodies and RNAi: The missing link? J RNAi Gene Silenc Int J RNA Gene Target Res 2: 105-106.
25. Kumar P, Ban HS, Kim SS, Wu H, Pearson T, et al. (2008) T cell-specific siRNA delivery suppresses HIV-1 infection in humanized mice. Cell 134: 577-586.

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