# Inflammation weakens HIV prevention

Thomas J Hope

**The presence of multiple inflammatory cytokines predicts the failure of a topical 1% tenofovir microbicide gel to protect women against HIV infection.**



Optimism is currently building in the efforts to develop therapies to prevent HIV acquisition. Large-scale clinical studies are revealing the approaches that work and others that are less likely to have an impact. Successes with pre-exposure prophylaxis (PrEP) are largely driving the current optimism with regard to these therapies. There are two types of PrEP, topical and systemic. Topical PrEP (microbicides) provides high concentrations of an antiretroviral (ARV) compound delivered locally[1]. The idea behind topical PrEP is to load mucosal cells with a drug that prevents HIV replication, with the goal of providing protection from HIV acquisition while avoiding systematic impact of the drug. The other approach is systemic PrEP, in which ARV compounds are introduced orally; this is similar to how HIV infection is treated. With systemic PrEP, therapeutic levels of drug are distributed throughout the body and can inhibit infection at mucosal sites. The current ARV formulations have few side effects, making systemic treatment of healthy but at-risk individuals a clinically acceptable option. This new reality, along with inefficiencies in the protection mediated by topical PrEP, has greatly reduced the interest of funding agencies in supporting early clinical development of topical PrEP formulations.

A large difference between the efficacies of topical versus oral PrEP has emerged from ongoing clinical trials[2]. Systemic PrEP can provide potent protection against HIV acquisition, especially in men, achieving levels of protection well over 90% in men who have sex with men[3]. Conversely, topical PrEP, such as gels and intravaginal rings, only provides modest protection in women, with protective levels typically less than 50% (ref. 4). Although the limited success of topical prep modalities has often been blamed on improper use and lack of compliance[5,6], biological reasons for the failure of topical PrEP have been emerging[7]. Trying to understand the underlying mechanisms that lead to this difference in efficacy of topical and systemic PrEP has become an important research focus. In this issue of *Nature Medicine*, McKinnon *et al.*[8] observe that inflammation can interfere with the effectiveness of topical PrEP. Their results will facilitate the development of improved PrEP formulations and approaches to refine future therapies for the prevention of HIV acquisition.

In the effort to understand why topical PrEP is not working as well as expected, McKinnon *et al.*[8] evaluated mucosal samples collected during a phase IIb clinical trial, known as CAPRISA 004, that evaluated the ability of a vaginally applied topical gel to prevent HIV acquisition. CAPRISA 004 was a double-blind, randomized, placebo-controlled clinical study in which South African women used a 1% tenofovir gel before and after intercourse to prevent HIV infection. This study was conducted by the Centre for the AIDS Programme of Research in South Africa (CAPRISA) at the University of KwaZulu-Natal. This was one of the first clinical trials to evaluate the ability of topical PrEP to prevent HIV infection. The 1% tenofovir gel reduced HIV infections by 39% relative to placebo[4]. Importantly, the clinical trial included the collection of mucosal samples. Mucosal samples are both challenging and expensive to collect and hence have not been obtained in many HIV prevention trials[9]. Evaluation of the CAPRISA 004 mucosal samples has revealed significant mechanistic insights into HIV acquisition and topical PrEP efficacy in women[4]. Assay of cervicovaginal lavage (CVL) samples revealed that elevated levels of certain inflammatory cytokines in the vaginal environment were associated with increased HIV acquisition in women[10]. Subsequent analysis of these samples revealed that certain bacterial species of the vaginal microbiome could metabolize tenofovir to reduce the potency of the gel formulation[7].

In this study, McKinnon *et al.*[8] analyzed the impact of genital inflammation on the effectiveness of the 1% tenofovir gel (**Fig. 1**). Genital inflammation was evaluated through determining the levels of mucosal inflammatory cytokines present in the CVL samples. Genital inflammation was considered present when there were at least three elevated inflammatory cytokines relative to average cytokine levels in all 774 women in the clinical trial[8]. In women with no genital inflammation and who adhered to advised drug use, the level of protection from the tenofovir gel was 75%. Conversely, women who adhered to the use of the tenofovir gel with genital inflammation had levels of protection of –10%. Therefore, even when the tenofovir gel was properly used, no protection was observed in the inflammatory mucosal environment. Clearly, these results indicate that there are biological factors influencing the ability of topical PrEP to prevent HIV acquisition in women.

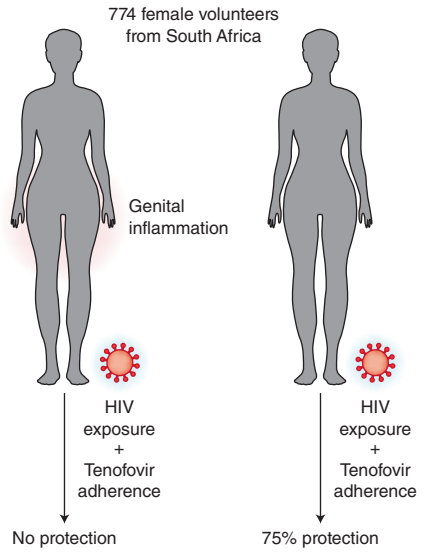


**Figure 1** Genital inflammation predicts the failure of a topical tenofovir gel microbicide to protect women against HIV infection. The detection of genital inflammation, as determined through the presence of elevated levels of at least three inflammatory cytokines in a CVL sample, was associated with failure of the 1% tenofovir gel microbicide to prevent HIV infection[8]. In women without genital inflammation, proper use of the gel before and after sex provided 75% protection. In contrast, no protection was observed in the presence of genital inflammation, even with adherence to gel usage.

Beyond providing insights into factors that influenced the efficacy of this tenofovir-based microbicidal gel product, these studies raise a series of questions about the mechanisms underlying transmission of HIV and protection from HIV transmission conferred by topical PrEP. It is not clear whether the inflammatory cytokines are generated in the upper or lower female reproductive tract (FRT) or whether

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they are released from focal sites of inflammation or systemically from one of the organs or tissues of the FRT. In addition, the underlying cause or causes of the high levels of inflammatory cytokines is also unclear, with sexually transmitted infections (STI), microbiome dysbiosis, contraceptive use, and sexual practices all having a potential impact. It is possible that all of these factors affect the physiology of local mucosal cells that are targets of HIV to some extent. Efforts are underway to better understand how these factors might lead to increased HIV acquisition.

To functionally inhibit HIV replication, tenofovir must be absorbed by the cell and modified by cellular enzymes to become tenofovir diphosphate (TFV-dp). TFV-dp is the active form of the drug and is trapped within the cell. TFV-dp must compete with intracellular concentrations of the nucleotide deoxyadenosine 5′-triphosphate to be incorporated into the forming DNA genome of HIV and block infection by inhibiting viral replication. This makes the function of this class of ARV compounds highly sensitive to intracellular nucleotide levels, which can vary greatly in each individual target cell. Therefore, a metabolically active cell with a high concentration of deoxyadenosine will require more TFV-dp to have the ability to block the virus[11]. It has been suggested that higher levels of deoxyadenosine in the FRT may make women less responsive to PrEP[11]. Therefore, the inflammatory milieu revealed by the presence of the indicating cytokines could be associated with increased deoxyadenosine levels in target cells at the local and systemic level, making the topical tenofovir gel less potent.

An additional mechanism that could explain the inhibitory effect of inflammation on tenofovir gel is an increase in the trafficking of HIV target cells into and within the mucosal tissue that is caused by or associated with the inflammatory cytokines. The drug is applied locally, which is likely to result in a heterogeneous gradient across the mucosa with high concentrations in the lumen side that decrease in concentration into the tissue. As target cells migrate into the area and become exposed to drug, they begin to accumulate TFV-dp. Therefore, it could take hours to days for a target cell moving into the drug gradient zone to accumulate enough TFV-dp to be protected from HIV infection. A high level of inflammatory cell infiltration and migration could lead to a large population of target cells without a protective level of drug being potentially exposed to the virus.

The important observation that the presence of increased inflammatory cytokines correlates with increased HIV acquisition and the failure of tenofovir topical gel is generating testable hypotheses that will advance the understanding of the mechanisms underlying HIV acquisition in women. Additionally, as described by the authors, it potentially provides a way to identify women that are at highest risk for HIV acquisition. An inexpensive and simple assay to evaluate the levels of inflammatory cytokines could determine the relative risk of infection and serve to inform those at highest risk to take the necessary precautions to prevent acquisition of the virus. This type of intervention could have a significant impact on decreasing the rate of HIV acquisition in women in South Africa and around the world.

COMPETING INTERESTS
The author declares no competing interests.

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# Predicting leukemia relapse

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**Mass cytometry of acute B cell lymphoblastic leukemias at diagnosis reveals intrapatient phenotypic heterogeneity and specific signatures that mimic cell developmental stage and predict future relapse.**

The classical model of clonal evolution proposed by Nowell[1] in 1976 states that cancer arises from a single cell that clonally expands upon the acquisition of somatic genetic changes. Although such clonal expansion is thought to result in a homogeneous neoplastic cell population, recent advances in genomics through next-generation sequencing and single-cell technologies propose a more complex and dynamic scenario. A developing cancer is subjected to differing evolutionary pressures, and once a neoplasm has clinically manifested, it frequently contains communities of cells with diverse molecular features. In fact, recent models of leukemia development consider dynamic clonal evolution as the driver of tumor initiation, disease progression and relapse[2].

B cell acute lymphoblastic leukemia (B-ALL) is one of the most common malignancies in childhood. Current treatments have led to striking improvements in survival, but relapse is still difficult to manage and represents the most frequent cause of death[3,4]. Current risk stratification of patients is based on clinical and genetic features at diagnosis as well as treatment response[3]. Recent deep genomics studies have characterized B-ALL genomic architecture and revealed that, in some patients, malignant subclones present at diagnosis seed future relapses post-therapy[5]. However, in spite of these advances, prediction of relapse, although possible to some extent, is not as effective as desirable.

In this issue of *Nature Medicine,* Good *et al.*[6], rather than focusing on genetic alterations, use a high-throughput single-cell phenotypic approach to understand intrapatient B-ALL heterogeneity and relapse and to identify markers that are predictive of future relapse.

The authors used single-cell cytometry by time-of-flight (CyTOF)[7,8] to simultaneously profile the expression of 35 B cell developmental proteins in bone marrow samples from 60 patients with B-ALL at the time of diagnosis and 5 healthy donors. To deeply characterize the B-ALL phenotype, the authors first analyzed healthy bone marrow to generate a classifier of developmental stage containing a total of 15 subpopulations, ranging from undifferentiated to mature B cells (**Fig. 1**). Subsequently, through superimposing each cell from each B-ALL sample onto the normal cell

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