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The case for transmissible antivirals to control population-wide infectious disease

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Infectious disease control faces significant challenges including: how to therapeutically target the highest-risk populations, circumvent behavioral barriers, and overcome pathogen persistence and resistance mechanisms. We review a recently proposed solution to overcome these challenges: antivirals that transmit by 'piggybacking' on viral replication. These proposed antivirals, termed 'therapeutic interfering particles' (TIPs), are engineered molecular parasites of viruses that are designed to steal replication resources from the wild type virus. Depriving viruses of crucial replication machinery, TIPs would reduce viral loads. As obligate parasites, TIPs would transmit via the same risk factors and transmission routes as wild type viruses, automatically reaching high-risk populations, and thereby substantially limiting viral transmission even in resource-poor settings. Design issues and ethical/safety considerations of this proposed intervention are discussed.

The problem: universal barriers to controlling infectious disease spread

Despite the enormous success of antimicrobial pharmaceuticals and vaccines, effectively controlling the spread of infectious diseases, whether bacterial, viral, or parasitic, has proved exceptionally challenging. Even for diseases with effective vaccines, the degree of control is dependent on achieving 'herd immunity', which normally exceeds 80% [1–3]. Unfortunately, there are significant logistical problems to achieving widespread vaccine or antimicrobial coverage and these are exacerbated by the fact that pathogens are dynamic (they evolve and transmit) whereas vaccines and pharmaceuticals are static. Specific challenges include: (i) the evolution of pathogen resistance, (ii) behavioral barriers such as poor adherence and associated increases in risky behavior, and (iii) arguably the most intractable for many diseases, the difficulty of targeting treatments to high-risk populations (core groups or 'superspreaders'; see Glossary) who are widely recognized as a major driving force underlying the circulation of many infectious diseases [4–7]. These factors lead to the most impoverished and disadvantaged groups, who are often the highest-risk groups, receiving the least access to interventions [8–11].

For HIV, small groups of commercial sex workers and their clients have long been known to drive HIV epidemic spread in larger populations, and recent phylogenetic analysis demonstrates that small groups of intravenous drug users (IDUs) establish core groups of 'superspreaders' that drive and sustain the larger HIV heterosexual epidemic in Eastern Europe [12]. Unfortunately, identifying and targeting these crucial high-risk populations is often not feasible in practice, in part due to disenfranchisement, self-concealment motivated by social stigmas, culture, and

Glossary

Antiretroviral therapy (ART): general name for a cocktail of small-molecule drugs which inhibit retroviral replication.
Behavioral disinhibition: an increase in risky behavior in response to treatment.
***Cis* elements:** necessary regions of the viral genome which are acted upon by *trans* element products to achieve viral replication. Examples include enhancers, promoters, and viral packaging signals.
Defective interfering particle (DIP): a mutant virus that contains significant genomic deletions or other debilitating mutations such that it is unable to replicate except when complemented by wild type virus replicating within the same cell.
gRNA: genomic RNA.
Incidence: the number of newly infected individuals in a population per unit time.
Multiplicity of infection (MOI): the average number of viruses infecting a single cell.
Prevalence: the proportion of a population infected with virus.
Reproductive ratio (R_0): conventional measure of replicative spread of a microorganism. For viruses, it is defined as the average number of new infected hosts generated by a single infected host, respectively, if the entire population is susceptible. If $R_0 > 1$, the infection spreads. If $R_0 < 1$, the infection dies out.
Superspreaders: for sexually transmitted diseases, these are core groups of individuals who engage in high-risk behaviors and drive a disproportionately large number of transmission events.
Therapeutic interfering particle (TIP): a synthetic DIP engineered to have missing key *trans* elements and an $R_0 > 1$, currently hypothetical.
***Trans* elements:** regions of the viral genome which encode gene products such as capsid proteins and transcription factors. *Trans* element products act upon *cis* elements.

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criminal barriers in these key populations [13–16]. The high cost and effort involved in identifying these cohorts has meant that – despite the huge potential benefits – targeting disease control measures to high-risk populations has remained an enormous barrier.

Although some models argue that widespread antiretroviral therapy (ART) campaigns – using highly effective drugs that lower HIV to undetectable levels – could halt the HIV/AIDS pandemic [17,18], there remains substantial controversy as to whether ART can succeed in reducing overall HIV transmission [19,20], especially when accounting for the presence of high-risk superspreader groups and non-adherence to treatment [19,21,22].

Based on these challenges, it is prudent to consider alternative and complementary approaches. We examine here a fundamentally different concept for therapeutic intervention, which would obviate the need to directly identify high-risk populations, would be robust to behavior changes, and could circumvent pathogen persistence and resistance mechanisms [21,23–25]. The concept relies on engineering TIPs that would spread between individuals to target high-risk groups autonomously. We argue that TIPs, if designed and implemented effectively, could be used to control HIV in regions such as sub-Saharan Africa, and would be compatible with antiretroviral therapy (ART), known to reduce HIV incidence and prevalence, or a hypothetical protective vaccine against HIV. We stress that TIPs remain hypothetical, and this work considers the risks and benefits of TIP intervention should successful engineering be accomplished. As such, this analysis can be seen as a first step in evaluating whether efforts to engineer TIPs should be undertaken.

A solution: engineering synthetic TIPs

Since the 1950s it has been recognized that many viruses, particularly RNA viruses, passaged *in vitro* at high multiplicities of infection (MOI), spontaneously generate defective interfering particles (DIPs), which are mutant viruses containing significant genomic deletions such that they are unable to replicate except when complemented by replicating wild type virus [26,27]. These DIPs interfere with wild type virus replication but do not eradicate wild type virus infections because they are dependent parasites of the virus. Given their inability to clear virus, DIPs have traditionally not been seen as viable antiviral therapy candidates. However, unlike conventional antiviral therapies, DIPs can transmit between individuals across populations [28]. As a result of their dependence on wild type virus replication, DIPs transmit along the same transmission routes and via the same risk factors as the wild type pathogen. If DIPs could be engineered to overcome safety concerns, the potential exists to effectively reach high-risk superspreader groups and overcome the deployment and adherence barriers inherent with conventional therapies and vaccines.

TIPs are based on the traditional concept of DIPs with the key difference that, unlike DIPs, TIPs would be synthetically engineered to have a basic reproductive ratio (R_0) > 1 [21,23,25]. For HIV, this $R_0 > 1$ would be achieved by designing into the TIP an expression asymmetry (denoted by a design parameter P) whereby the TIP would generate increased levels of genomic RNA (gRNA) relative to wild type HIV gRNA in the infected cell [21]. As a result, TIP gRNA would stoichiometrically outcompete wild type virus gRNA for intracellular viral resources generated by the wild type virus, such as packaging elements, and this would enable TIPs to transmit more efficiently than wild type virus from the infected cell. In contrast to gene therapy approaches [29–31], theoretical analysis argues that TIPs should not encode antiviral or therapeutic elements that specifically target viral processes because such antiviral elements would reduce the viral resources TIP relies upon [25].

To construct efficient TIPs that satisfy the $R_0 > 1$ criterion, expression of one or more of the virulence elements (i.e., *trans* elements) of the pathogen must be abrogated in the TIP. Therefore, TIPs would inherently lack a subset of structural and envelope genes required to self-replicate, but they must retain all requisite *cis* signals. By parasitizing these viral resources intracellularly, TIPs would have the potential to decrease disease progression *in vivo*, and this is likely to lead to reduced disease transmission at a population scale (Box 1).

Transmissibility would enable TIPs to concentrate automatically in high-risk groups

The efficacy and robustness of a TIP intervention would ultimately arise from the proposed unique and defining ability of TIPs to transmit between hosts. The theoretical capacity of TIPs to transmit seems plausible and appears to be independent of the transmission biology. Computational models, with either 'founder' virus transmission or virus 'bottlenecking' post-transmission, show that TIPs would transmit efficiently through populations [21,23]. Because TIPs would transmit along the same transmission routes as HIV, they would autonomously target the highest-risk groups, which would be highly likely to be already infected with HIV owing to their high-risk status. Unlike ART or a hypothetical HIV vaccine, which require treatment of at least a critical proportion of the at-risk population to curb an epidemic, deploying TIPs to only a few infected individuals could lead to a substantial decrease in HIV prevalence if the proposed TIP transmission is efficient [21,23].

Current prevention and treatment approaches also face the challenges of poor adherence and behavioral disinhibition (i.e., risk compensation) [32], and can result in increases in risk behavior and may actually increase HIV incidence [33]. However, because TIPs would replicate conditionally, they would transmit ('piggyback') with the same risk-factor profile as the wild type pathogen, thereby effectively circumventing the disinhibition problem [33].

One potential caveat for future TIP intervention is that any intervention against HIV is likely to be administered in the context of the existing 'standard of care': ART. ART halts HIV replication and transmission, upon which TIPs would be dependent, and it would therefore seem that TIPs would face a major implementation problem. Although effective ART would halt TIPs within an individual, this is not the case at the population scale because TIPs would concentrate in highest-risk groups, whereas ART and vaccines inherently target lower-risk segments of the

Box 1. The TIP strategy

TIPs 'piggyback' to impact disease spread from the single cell scale to the epidemiological scale. At the single cell scale (Figure I, top right), dually infected cells would produce both HIV and TIP genomes. TIP genomes, being deletion mutants of wild type virus, would replicate more rapidly and reach higher concentrations than HIV in dually infected cells and would stoichiometrically outcompete wild type virus for *trans* element products (e.g., viral proteins) required by HIV for replication and packaging, and would thus interfere with HIV replication. At the individual patient level (Figure I, lower right), dually infected cells would produce more TIPs than HIV. The new TIPs would then infect additional target cells, pre-empting these cells to produce TIPs preferentially once they become infected by HIV. In this way, TIPs would reduce HIV viral load and would generate a TIP viral load in the patient. At the epidemiological scale (Figure I, left), susceptible individuals would acquire HIV, would become HIV-infected, and would ultimately progress to AIDS in ~10 years (higher viral loads lead to faster progression to AIDS, and lower viral loads delay progression). Owing to reduced HIV viral load, individuals dually infected with HIV and TIPs would transmit less HIV, and instead transmit TIPs, and would progress more slowly to AIDS.

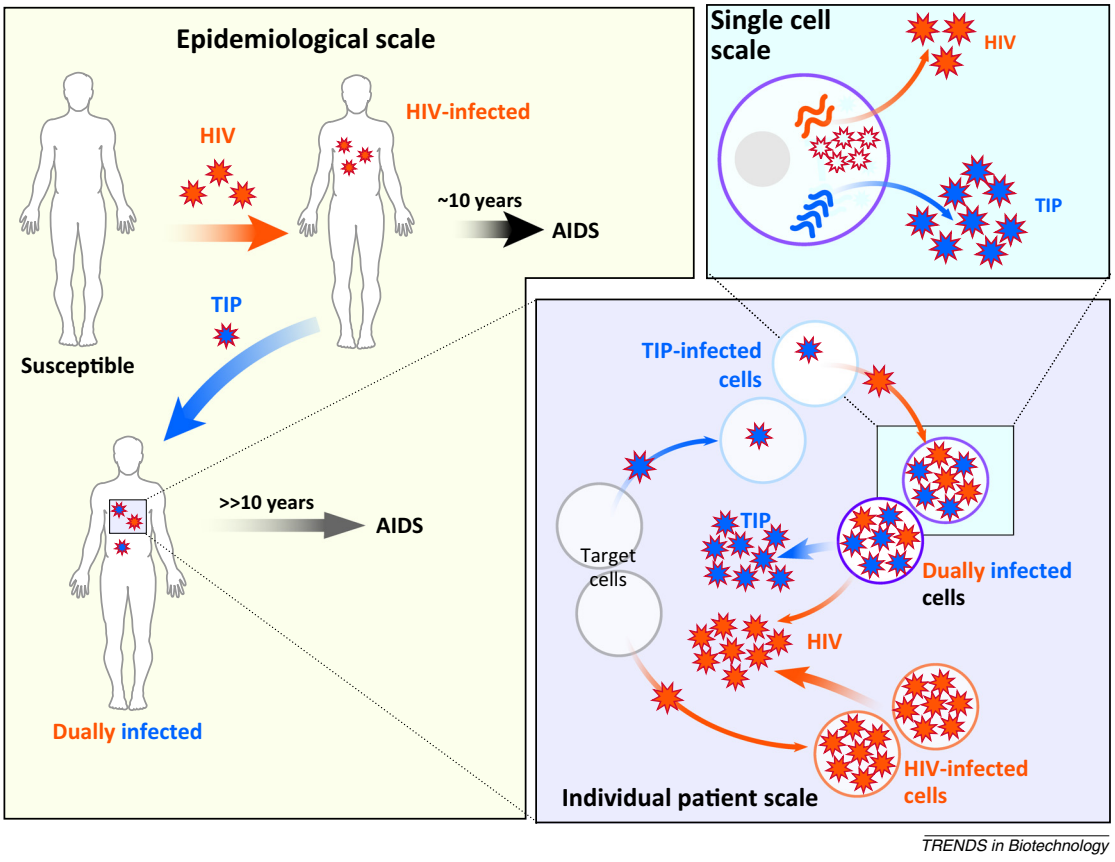

Figure I. Schematic of proposed TIP replication and transmission at the single cell scale (top right panel), individual patient scale (bottom right panel), and epidemiological scale (left panel). Orange and blue stars indicate HIV viruses and TIPs, respectively.

population. Consequently, TIP intervention could maintain efficacy, and even synergize with ART to reduce AIDS deaths more effectively than ART alone. Thus, ART would not interfere with TIP intervention at the population scale, and TIPs could be used as a powerful complement to ART and pharmaceutical treatments in general.

Risk–benefit analysis

All medical interventions carry inherent risk. Interventions that enter into clinical practice are those where the benefits have been determined to outweigh the risks. Risk–benefit analysis is thus an essential aspect of any medical innovation. As detailed above, the TIP approach offers several potential benefits over conventional pharmaceutical-based therapies and vaccines. We describe carefully here the risks of proposed TIPs compared to the benefits that such TIPs would offer. The key safety issues with all lentiviral vectors such as TIPs are threefold: (i) the oncogenic potential of integrating viruses, (ii) the potential generation of replication-competent lentivirus (RCL), and (iii) the possibility of generating a more severe strain of HIV-1 or increasing overall HIV transmission. The TIP approach also carries unique safety and ethical concerns associated with introducing an intervention that would transmit and evolve, even in the limited, HIV-dependent fashion of TIPs. Importantly, there are established precedents for transmitting therapies: many live attenuated vaccines transmit between individuals, which for some of these vaccines is a recognized benefit that outweighs the associated safety concerns. Overall, if rigorous testing in animal models and humans demonstrates that the efficacy benefits of successfully engineered TIPs outweigh the safety risks, then TIPs will need to be considered seriously as an intervention.

Efficacy precedents for a TIP strategy

Oral polio vaccine (OPV) is a live attenuated vaccine chosen by the World Health Organization (WHO) for the worldwide polio eradication effort [34]. Although OPV can revert to cause serious disease, the adoption of OPV (over the safer inactivated or subunit polio vaccines) is due to two key factors: low cost, and the ability of OPV to spread, in a limited fashion, between individuals. This OPV transmission phenomenon is a key recognized benefit, which, together with cost, outweighs the serious safety concerns associated with OPV (http://www.polioeradication.org/Polioandprevention/Thevaccines/Oralpoliovaccine(OPV).aspx).

Unlike OPV, TIPs would conditionally replicate, and only in individuals who are already infected. Thus, TIPs would encode an inherent safety-lock: unlike live attenuated vaccines, TIPs would require the pathogen to mobilize, and could only 'revert' in already infected individuals. This type of reversion is akin to a loss of efficacy of a drug or vaccine. Moreover, it appears theoretically feasible to construct evolutionarily robust TIPs that maintain interference with HIV [23,24].

Another precedent for TIP transmission is found with Dengue fever virus (DENV), where naturally occurring DENV DIPs appear to attenuate infection [35,36]. Importantly, these natural molecular parasites of DENV transmit through human populations in Southeast Asia [28].

Safety considerations and risk assessment

One concern that has previously been raised for other HIV interventions is that reduced virulence could increase overall transmission because infected individuals would have a prolonged infectious state. This transmission–virulence tradeoff is frequently cited as an example of why extremely virulent pathogens, such as Ebola, exhibit localized epidemics that do not span vast multinational regions. Phylodynamic modeling led one group to infer that the presence of DENV DIPs increases DENV incidence, but this result is predicated upon an assumed parabolic relationship between viral load and transmissibility, which has yet to be measured [35]. Nevertheless, for HIV, extensive data show that reducing virulence does not lead to increased incidence, and populations in which individuals with lower viral loads and extended transmission lifetimes actually exhibit lower incidence of HIV [37–39].

There is the potential for any transmissible therapy, such as the proposed TIPs or OPV, to be viewed with suspicion as a 'new pathogen.' This perception could hamper efforts to reach the target population. The OPV campaign has used education and outreach to allay such fears [34], and we feel that a similar effort would be successful for TIPs.

Oncogenic concerns are minimized: TIPs would not require or target stem cells

Lentiviral vectors carry the risk of insertional oncogenesis, and stable lentiviral integration into host genomes can lead to expression changes in proto-oncogenes near the insertion site [40,41]. Because TIPs would be lentiviral vectors, they could carry the same risks. However, multiyear gene therapy trials in humans have now shown that lentiviral and retroviral vectors have excellent safety profiles [29,30,42,43]. Nevertheless, understandable concerns remain regarding retroviral gene therapy approaches, in part owing to severe complications of insertional mutagenesis leading to lymphoproliferative disease (leukemia) in a well-known gene therapy trial in France [44]. Insertional mutagenesis was a particular concern when highly sensitive hematopoietic bone-marrow stem cells were transduced with murine leukemia virus (MLV)-based retroviral vectors [40,41]. Importantly, the TIP approach would not be dependent on transducing hematopoietic stem cells (or any type of bone-marrow progenitor cell) and would depend only on transducing or infecting the cell type in which the virus primarily replicates: terminally differentiated T lymphocytes in the case of HIV. Gene therapy into peripheral blood lymphocytes has been very successful [45] and no insertional mutagenesis leading to lymphoproliferative disease was observed over decades [43]. Although B cell leukemias exist in HIV patients, HIV does not infect (or integrate) in B cells; immune misregulation is thought to drive these leukemias [46]. Thus, concerns about oncogenesis with TIPs would be substantially minimized.

Recombination concerns are minimized: recombination would occur only in already infected individuals

Retroviruses are pseudodiploid and carry two strands of gRNA which can recombine during reverse transcription. Recombination occurs when the reverse transcriptase enzyme undergoes template switching between the two different gRNA strands delivered in a single retroviral particle to the infected cell.

A potential concern is that recombination between TIPs or between TIPs and HIV could lead to generation of 'self-replicating TIPs' (self-replicating TIP/HIV or TIP/TIP chimeras). How likely is this to occur and what are the consequences? Generation of 'self-replicating TIPs' via recombination could only occur in individuals already infected with HIV because recombination requires active infection, as outlined above. Recombination between TIPs alone, such as in an HIV-negative individual during initial inoculation with TIPs, or in a dually-infected individual, would not lead to a self-replicating virus. Recovery of a replication-competent virus would be prevented by a design feature of TIPs – the severe deletions of HIV *trans* elements. For HIV-infected individuals, murine models [47,48] and human trials [29,30,42] show no evidence of recombination between wild type HIV and shorter lentiviral-therapy vectors, suggesting that, if recombination does occur, the resulting progeny are less fit than wild type HIV. Moreover, a recent theoretical study showed that recombination between DIPs and HIV-1 is unlikely to be evolutionarily conserved [23,24]. Thus, we feel that recombination between TIPs or between TIPs and HIV would not be a major concern.

Another possibility that must be considered is the theoretical potential of TIPs to recombine with endogenous retroviruses to reconstitute replication competent lentivirus (RCL) in HIV-1 naïve patients. Although this has been observed in a laboratory setting with murine gammaretroviral vectors, there are no reports in the lentiviral vector

clinical trial literature, likely due to the lack of homology between lentiviral vectors and endogenous retroelements [49]. Overall, lentiviral vectors, including a conditionally replicating vector, have an excellent safety record [29,30,50,51].

There is also the possibility that TIPs might recombine and acquire a genetic element that upregulates HIV expression. In this proposed scenario, TIPs would also upregulate their own expression, and the element would therefore not be selected against. Theoretical studies examining this scenario show that at the individual-patient level, TIP-mediated upregulation of HIV would lead to increased TIP viral loads, which would ultimately generate even lower HIV viral loads and a lower modeled HIV population prevalence [21]. Thus, TIPs may be subject to competing selection pressures at multiple scales, which would limit the potential for evolutionary breakdown of TIP therapies.

Ethical considerations

Ethical concerns all center on iatrogenic potential: is the potential of harm from therapy outweighed by the benefits? In other words, risk–benefit analysis also serves as the basis for the ethical considerations: if the benefits outweigh the risks there is ethical obligation to proceed with an intervention. Even in the case of OPV, with its risk of reversion and disease, the benefits of this vaccine led the WHO to adopt OPV.

Although the notion of a transmissible therapy – even a potential TIPs therapy, which would only transmit in a limited fashion between already infected individuals – may raise consent issues, many of these ethical issues have already been addressed for live attenuated vaccines such as OPV. The ethical considerations surrounding transmission for TIPs would be more limited than for live attenuated vaccines because live attenuated vaccines transmit indiscriminately whereas TIPs would only transmit via the same high-risk routes as the pathogen.

Furthermore, feasibility studies would be necessary to test the efficacy of TIPs as a transmissible therapy. Structured treatment interruptions, in which HIV-positive patients are temporarily taken off ART to test the effect of a new therapy, could be used to determine if TIPs would reduce viral load [29,30]. In addition, serodiscordant couples (in which one partner is HIV-positive and the other is HIV-negative) would be a valuable cohort to follow to determine properties of TIPs transmission and the effect of TIPs on HIV transmission [52].

Concluding remarks and future perspectives

Infectious disease control remains exceptionally challenging owing to the formidable universal barriers of how to target the highest-risk populations, pathogen persistence and resistance, and behavioral issues in the host population. By harnessing the replicative aspect of pathogens that create barriers for existing therapies, TIPs present a novel concept for overcoming these barriers. The TIP concept may have broad application to many viral infectious diseases and would represent a new paradigm for disease control.

For HIV/AIDS, epidemiological impact projections for a hypothetical TIPs strategy show that deploying TIPs as a therapy to even a few individuals who are already infected may, if successful, have the potential eventually to reduce the prevalence in sub-Saharan Africa to below epidemic levels [21,23].

The arguments herein represent a first step towards motivating research into transmissible therapies, rather than a proof of efficacy. The TIP approach does not argue that ART campaigns or vaccine trials need to be halted for HIV. In fact, on the contrary, complementary use of ART and TIPs may synergize to reduce HIV/AIDS burden. Further, dormant TIPs might even act as a backup therapy to reduce HIV viral load by reactivating in the event of ART failure.

The molecular, epidemiological, and ethical bases of using TIP intervention against pathogens will require extensive study, but projections argue that TIPs may offer a unique strategy for targeting both high-risk and hard-to-reach populations, overcoming behavioral barriers, and circumventing mutational escape to achieve indefinite disease suppression of HIV, and possibly other pathogens, in resource-limited settings.

Disclaimer statement

T.N. and L.S.W. are named as inventors on an International Patent Application applied for by the J. David Gladstone Institutes related to therapeutic interfering particles.

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