Over the past two decades, HIV resistance to antiretrovirals (ARVs) has risen to high levels in the wealthier countries of the world able to afford widespread treatment. We have gained insights into the evolution and transmission dynamics of ARV resistance by designing a biologically complex multi-strain network model. Using this model, we traced the evolutionary history of ARV resistance in San Francisco and predict the future dynamics. Using classification and regression trees, we have identified the key immunologic, virologic and treatment factors that increase ARV resistance. Our modeling shows that 60% of the currently circulating ARV-resistant strains in San Francisco are capable of causing self-sustaining epidemics as each individual infected with one of these strains can cause on average more than one new resistant infection. It is possible that a new wave of ARV-resistant strains that pose a significant threat to global public health is emerging.

HIV resistance to antiretroviral drugs (ARVs) is causing serious clinical and public-health problems throughout the USA and Europe. HIV strains began to acquire resistance in 1987 when ARVs were introduced as therapies for HIV-infected individuals (1). Since then, a multitude of drug-resistant strains have evolved that differ considerably in their susceptibility to three major classes of ARVs: Nucleoside Reverse Transcriptase Inhibitors (NRTIs), Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) and Protease Inhibitors (PIs). These drug-resistant strains are now being transmitted to individuals who have never received ARVs; i.e., transmitted drug resistance (TDR) has arisen. TDR is reported to range between 8-22% in many HIV-infected communities in resource-rich countries and if it continues to increase, the effectiveness of therapeutic regimens, as well as efforts to control the HIV pandemic, will be compromised.

We have developed a theoretical model (the amplification cascade model) to help understand and predict the evolutionary dynamics of complex transmission networks composed of multiple ARV-resistant strains. We calibrated and parameterized the model to represent the HIV epidemic in San Francisco in the community of men who have sex with men (MSM), where levels of TDR are already high (~13%) (2). The model reproduced the observed dynamics and evolution of transmitted resistance in this city over the past 20 years. Here, we have used the model first to predict the future evolutionary dynamics of TDR; second, to determine whether any of the currently circulating ARV-resistant strains are capable of generating self-sustaining epidemics; and third, to identify the key drivers that generate high levels of TDR. We have also discussed the implications of our results for resource-constrained countries where ARV treatment programs are being rolled-out.

All the published HIV transmission models of ARV-resistance are based on simple biological assumptions and can only track one resistant strain (3–8). Our amplification cascade model captures biological complexity by generating a dynamic network composed of multiple ARV-resistant strains. We modeled the multi-strain network in San Francisco by classifying ARV-resistant strains into seven categories; each category was defined based upon the specific class of drugs the strain was resistant to (NRTIs, NNRTIs or PIs) and the level of resistance (single, dual or triple class) (Fig. 1A). Single-class resistance was to NRTIs, NNRTIs or PIs and the level of resistance (single, dual or triple class) (Fig. 1A). Single-class resistance was to NRTIs, NNRTIs or PIs. Dual-class resistance was to NRTIs and NNRTIs, NNRTIs and PIs, or NRTIs and PIs. Triple-class resistance was to all three. Each class of ARVs contains several drugs [fig. S3 and table S1 in the Supplementary Online Material (9)]. In our modeling framework, if a strain is classified as resistant to a certain class of ARVs, it signifies the strain is resistant to at least one drug in that class.
We modeled treatment effects by specifying treatment regimens and then assessing the effects of these regimens on infectivity and the probability of developing resistance. Treated individuals receive a regimen to which their virus is sensitive; hence we assume treated individuals either achieve complete or partial viral suppression. We consider patients who achieved complete viral suppression to be uninfected and incapable of developing resistance. Patients who only achieve partial viral suppression retain some degree of infectivity and are capable of developing resistant strains. In the model, when individuals experience treatment failure (which is usually determined by viral rebound) they can be switched to new drugs either in the same class or in a new class. For example, if a patient (in the model) is on a regimen containing zidovudine (NRTI), lamivudine (NRTI) and nelfinavir (PI), and develops resistance to nelfinavir, they could be switched to another PI (e.g., indinavir). The model includes a matrix that specifies the rates at which strains develop resistance; therefore, strains are directly linked through the acquisition and amplification of resistance.

Resistant and wild-type strains were assumed to compete to transmit HIV to uninfected, at-risk MSM. In the model, these competitive interactions are mediated through strain-specific infectivity; the greater the infectivity, the higher the probability that the strain will be transmitted. We ascribed a competitive advantage to wild-type strains by assuming they are always more infectious than resistant strains. Furthermore, based on available competitive fitness assays, replication capacity assays and patterns of developed resistance, we assumed NNRTI-resistant strains were more transmissible than NRTI-resistant strains, which in turn were more transmissible than PI-resistant strains (10, 11). In addition, we assumed, based on the available data, the transmissibility of virus strains decreased as the number of classes of resistance increased (12). Once an individual becomes infected with a wild-type or resistant strain, the model tracks viral dynamics, and consequently infectivity, through four stages of disease progression: first, primary infection; second, not yet eligible for ARVs (i.e., CD4 count > 350 cells/microL); third, eligible for ARVs (i.e., CD4 ≤ 350 cells/microL) but not currently undergoing ARV treatment; and fourth, ARV treatment. The 33 equations that specify the model, as well as a more detailed description of the structure, are given in (9). Parameter estimates are discussed in Section 2 and tables S2–S11 (9). The model can be extended to include any number of additional drug classes, such as integrase inhibitors, co-receptor blockers and fusion inhibitors, as they are introduced into new therapeutic regimens.

Before making predictions, we used the model (coupled with an uncertainty analysis) to reconstruct the evolution and transmission dynamics of the network of ARV-resistant strains (13). We calibrated the model, using Monte Carlo filtering techniques, to match the epidemiological conditions in San Francisco in 1987 when ARVs were first introduced [Section 3 and table S12 (9)]. It has been estimated almost half of the MSM community was infected with HIV by the late 1980’s (14, 15). After calibration we used the model to simulate, from 1987 to 2008, the evolutionary dynamics of a network of ~4,000 resistant strains; where each strain differed in drug susceptibility and infectivity. The history of ARV therapy in San Francisco can be divided into four eras spanning two decades (16) [fig. S3 and table S1 (9)]. Different regimens were used in each era. We modeled the specific regimens that were available in each era by using data on the: proportion of patients achieving viral suppression [tables S6-S9 (9)], degree of reduction in viral load in partially-virally-suppressed patients [tables S2 and S5 (9)], rate of development of resistance in treated patients [tables S6-S9 (9)], and treatment-induced increase in survival time [table S10 (9)]. Since usage of ARVs has increased over the past two decades we modeled era-specific treatment rates [table S4 (9)].

The model reproduced and explained the observed evolutionary dynamics of the network of ARV-resistant strains over the four treatment eras (Fig. 1B). The first era began in 1987 when AZT (an NRTI) was introduced as monotherapy. AZT was used by a high proportion (36-68%) of MSM in San Francisco (17–19). Since it was ineffective in suppressing viral loads (19), single-class resistance to NRTIs arose quickly (1). In 1992, the second era began when dual therapies (based upon two NRTIs) were introduced. These therapies were substantially more effective than monotherapies and achieved 30-60% viral suppression (20, 21). Single-class resistance to NRTIs decreased, but dual-class resistance quickly developed since many individuals had previously developed resistance to AZT. In 1996, the third era (early HAART) began when NNRTIs and PIs were used in triple-therapy regimens. Resistance to PIs was slow to emerge and has only risen to low levels because multiple mutations are necessary to develop resistance to most drugs in this class (22). By 2001, more effective triple therapies (characterized by dual PIs combined with NRTIs) were developed, marking the beginning of the fourth era (modern HAART). During this recent era the overall level of TDR appears to have stabilized (2); notably, the model-generated network also exhibits this behavior (Fig. 1B). Recent empirical data from San Francisco indicate transmission of single-class resistance is high, dual-class is moderate and triple-class is low. In addition, studies indicate transmission of NNRTI resistance is greater than NRTI resistance, which is greater than PI resistance. The model-generated transmission network shows these same patterns (Fig. 1C and 1D). Notably, our modeling estimates the overall level of TDR in
2008 to be 14% (median: IQR 11.4-16.5%) (Fig. 1C), this is in extremely close agreement with empirically-derived estimates of 13%-16% (2).

After reconstructing the historical epidemiology up to 2008, we simulated the amplification cascade model for a further five years to predict the levels of TDR in 2013. Our simulations revealed that resistance to single-class NRTIs and PIs will remain at current levels, but NNRTI resistance will increase (Fig. 2A). Regression analysis determined the degree of increase in NNRTI resistance will depend (p < 0.05) on the proportion of patients (who are infected with wild-type strains and being treated with a regimen of two NRTIs and one NNRTI) who achieve viral suppression (Fig. 2B). This proportion depends on the efficacy of the regimen and adherence to it; thus, if only 70% are virally suppressed, NNRTI-resistance could increase by more than 30% (Fig. 2B). This increase is predicted to be mainly due to transmission from untreated individuals infected with NNRTI-resistant strains who are in either the acute or chronic stage of infection.

The value of a strain’s control reproduction number $R_c$ specifies the average number, based on the probability the individual is treated, of secondary HIV infections an individual generates during their entire infectious period. $R_c$ is a measure of a strain's transmission potential. A strain is capable of generating a self-sustaining epidemic if $R_c > 1$. The $R_c$’s of the currently circulating ARV-resistant strains in San Francisco vary considerably (Fig. 3A). However, strains fall into three mutually exclusive groups (Fig. 3B) [Section 4 (9)]. Almost a quarter (24%) of the strains (Fig. 3B) cause less than one new infection ($R_c < 1$) and will eventually be eliminated. Although other strains (Fig. 3B) also cause, on average, less than one new infection ($R_c < 1$), they will continue to be transmitted because they evolve greater levels of resistance. Notably, we estimated that 60% of resistant strains have an $R_c > 1$ (Fig. 3B). Approximately 75% of these resistant strains have single-class resistance to NNRTIs, and 20% have dual-class resistance to NNRTIS and NRTIs. Although all have the potential to cause self-sustaining epidemics of resistance, they are all less infectious than the wild-type strains in San Francisco (Fig. 3C).

Similar trends for TDR to those observed in San Francisco, and predicted by our model, have been documented in other cities in the USA and Europe that have analogous histories of ARV therapy. Potentially NNRTI-resistant strains similar to those we have identified in San Francisco may be increasing elsewhere. Although the NNRTI-resistant strains that we have identified are causing the rising wave of NNRTI resistance, they are unlikely to lead to self-sustaining epidemics in San Francisco or other communities in resource-rich countries as new drugs will continue to become available. However our results may have significant implications for HIV treatment programs in resource-constrained countries where second-line regimens are not generally available. NNRTI-resistant strains are already evolving in many of these countries because their first-line regimens are based on two NRTIs plus one NNRTI. Our current predictions have been obtained by modeling the evolution of resistance in individuals infected with subtype B strains. Subtype B accounts for ~12% of worldwide infections (and persons with subtype B are the most ARV-experienced), but 50% of prevalent HIV infections and 47% of all new HIV infections world-wide are due to subtype C (23). Although information is limited, preliminary data suggest that treatment response and resistance patterns for subtype C are similar to subtype B (24). These data suggest our results are likely to be generalizable to an epidemic of HIV-1 resistance among individuals infected with HIV-1 subtype C and NNRTI-resistant strains with $R_c > 1$ could emerge in resource-constrained countries. If the $R_c$ of the wild-type strains is reduced below one, as could occur by using a universal testing and treatment strategy (25), self-sustaining epidemics of NNRTI-resistant strains could arise [Fig. 3B, Section 5 and fig. S5 (9)].

Current levels of TDR, as well as the biological composition of the complex multi-strain network, have emerged from two decades of treatment. To identify the key drivers of ARV resistance, we constructed classification and regression trees (CART) (26) using the twenty year data set (1987 to 2008) generated during the uncertainty analysis of the amplification cascade model. To build trees, we used the model’s estimated level of TDR for 2008 as the response variable and the model’s 50 parameters as predictor variables [Section 6 (9)]. The optimal tree revealed the hidden hierarchical structure of the data (Fig. 4). Key drivers of TDR are the predictor variables with the highest importance scores (IS) [table S13 (9)]. The most important driver (IS=100) is the average time (at the population level) it takes for CD4 cell counts in infected individuals to fall below 350 cells/microL ($V^{-1}$) (Fig. 4). TDR was significantly higher (>15%) when CD4 counts fell to this threshold within ~6 years than when counts fell more gradually [Fig. 4 and fig. S6A (9)]. This occurred because faster immunological deterioration led to increased treatment rates and accelerated the acquisition of resistance; hence, TDR increased as $V^{-1}$ decreased.

A high proportion of the transmission of wild-type strains over the past twenty years has occurred from asymptomatic individuals with a CD4 count > 350 cells/microL [fig. S6B (9)]. Consequently, the infectiousness of strains in asymptomatic individuals ($\alpha^{tf}$) has been the second key driver of TDR (IS = 73) (Fig. 4); infectivity is defined in terms of the per sex act probability of transmitting HIV.
These results can be understood in terms of classical competition theory (27); the most infectious wild-type strains had the greatest advantage over resistant strains and hence caused the lowest levels of TDR. A recent review of empirical estimates of the per-sex-act transmission probability indicate $\alpha_{1}^{H}$ is likely to be greater than 0.0024 (28). The tree (Fig. 4) reveals that if wild-type type strains had been less infectious (specifically, $\alpha_{1}^{H} \leq 0.0024$) it would have been probable ($P=0.71$) that TDR in San Francisco would be even higher than the current level [fig. S6C (9)].

We found the infectiousness of strains under treatment pressure ($\alpha_{1}^{T}$) to be the third key driver of TDR (IS = 60) (Fig. 4). This driver represents the probability that an individual who is receiving current ARV regimens transmits HIV during one sex act. In contrast to our previous finding for $\alpha_{1}^{H}$, TDR was significantly higher (> 15%) when wild-type strains were more infectious ($\alpha_{1}^{T} > 0.0015$) than when they were less infectious ($\alpha_{1}^{T} \leq 0.0015$) (Fig. 4). This paradoxical result cannot be understood in terms of classical competition theory (27). It occurred because the effect of evolution on network dynamics was greater than that of competition. Under treatment pressure, the most infectious wild-type strains ($\alpha_{1}^{T} > 0.0015$) tended to evolve into the most infectious resistant strains; $\alpha_{1}^{T}$ only had a minor effect on competition since treated individuals were relatively unimportant in transmitting wild-type strains [fig. S6D (9)]. The value of $\alpha_{1}^{T}$ can be translated into viral load (Section 2 and fig. S2 (9)); a value of 0.0015 corresponds to a viral load of 20,000 copies/mL. Effective therapies used in recent years have reduced viral loads in patients infected with wild-type strains to well below 20,000 copies/mL (29), indicating that $\alpha_{1}^{T}$ is (and was) significantly less than 0.0015. Given these effective treatments, our tree shows it is highly unlikely ($P=0.78$) that TDR in San Francisco could have risen to more than 15% by 2008 (Fig. 4).

Our CART analysis also identified four other parameters that are important drivers of TDR, including the relative transmissibility of strains with single-class resistance to NRTIs ($\beta_{2}$) (IS=51), the degree of viral suppression in patients who are infected with wild-type strain and not completely virologically suppressed ($\gamma_{1}$) (IS=45), the relative transmissibility of strains with dual-class resistance to NRTIs and NNRTIs ($\beta_{3}$) (IS=40), and finally the degree of viral suppression in patients who are infected with strains that have single-class resistance to NNRTIs and are not completely virologically suppressed ($\gamma_{3}$) (IS=39). None of the 43 other predictor variables were found to be important (IS < 30). The tree shows that TDR has remained below 15% because of specific immunologic, virologic and treatment factors operating in San Francisco (Fig. 4).

The amplification cascade model can be recalibrated and reparameterized to assess the dynamics of networks of ARV-resistant strains of HIV in any setting where ARVs are available. Here we have applied it to San Francisco. We have shown a complex network of HIV strains has arisen in this city, due to two decades of sequential selection for resistance; first with single agents, then dual agents and more recently a combination of multiple-class agents. By designing a biologically complex multi-strain network model, we have obtained important insights into the otherwise hidden dynamics of drug-resistant strains of HIV. We have identified the key immunological, virological and treatment variables, as well as the hierarchical interactions among these variables, which have had a key role in driving resistance. Our results have shown that effective treatments have prevented TDR from increasing to greater than 15% in San Francisco. However, our modeling shows the network is continuing to evolve. Disturbingly, we found the majority of the resistant strains currently being transmitted in this city are capable of causing self-sustaining epidemics and have estimated that an individual with a NNRTI-resistant strain can cause on average more than one new infection. We predict a wave of NNRTI-resistant strains will emerge over the next five years in San Francisco due to transmission from untreated individuals. Our results also have implications for resource-constrained countries where first-line regimens are based on NNRTIs. If the resistant strains we have identified in our analyses evolve in these countries, they could significantly compromise HIV treatment programs. Consequently, currently circulating NNRTI-resistant strains in San Francisco pose a great and immediate threat to global public health.

References and Notes
9. Materials and methods are available as supporting material on Science Online.
Fig. 1. (A) Schematic diagram of the multiple pathways in the amplification cascade model by which strains can acquire resistance. Strains may develop single-class resistance to NRTIs (blue; denoted N), single-class resistance to NNRTIs (red; denoted NN), single-class resistance to PIs (purple; denoted PI), dual-class resistance to NRTIs and NNRTIs (green; denoted N+NN), dual-class resistance to NRTIs and PIs (orange; denoted N+PI), dual-class resistance to NNRTIs and PIs (yellow; denoted NN+PI), or triple-class resistance (brown; denoted Triple). Wild-type strains are shown in gray. There are six possible paths by which strains can develop triple-class resistance. (B) Representative simulation generated by the amplification cascade model to show the evolution of ARV resistance in the MSM community in San Francisco. The same color coding holds as in A. (C) Estimated levels of TDR in 2008 using Monte Carlo simulations from the uncertainty analysis of the amplification cascade model. Single-class resistance is 8.5% (median: Inter-Quartile Range (IQR) 6.8-9.8%) (red), dual-class resistance is 4.5% (median: IQR 3.5-5.8%) (green) and triple-class resistance is 1.0% (median: IQR 0.7-1.3%) (blue); overall levels of TDR are in black. (D) Boxplots of estimated levels of TDR in 2008 based on Monte Carlo simulations from the uncertainty analysis of the amplification cascade model. The same color coding holds as in A. Horizontal black lines represent medians, boxes show Inter-Quartile-Range.

Fig. 2. (A) Predictions showing transmission of strains that are resistant to NNRTIs will increase in San Francisco over the next five years. Predictions were made using Monte Carlo simulations from the uncertainty analysis of the amplification cascade model. Red lines show no increase in NNRTI or NRTI resistance over the next five years. Predictions were made using Monte Carlo simulations from the uncertainty analysis of the amplification cascade model. Red lines show no increase in NNRTIs or NRTIs over the next five years. (B) Predicted increase in the level of transmitted NNRTI resistance in San Francisco over the next five years as a function of the proportion of patients (who are infected with wild-type strains and being treated with a regimen of two NRTIs and one NNRTI) who achieve viral suppression. Predictions were made using Monte Carlo simulations from the uncertainty analysis of the amplification cascade model.

Fig. 3. (A) Boxplots of the control reproduction numbers ($R_c$) for all seven categories of ARV-resistant strains in the amplification cascade model. The same color coding holds as in Fig. 1A. Horizontal black lines represent medians, boxes show Inter-Quartile-Range. (B) Classification of ARV-resistant strains into three mutually exclusive groups based on
their transmission potential: resistant strains that cause less than one new infection ($R_c < 1$) and will eventually be eliminated (blue), resistant strains that cause less than one new infection ($R_c < 1$) but will continue to be transmitted (green), and resistant strains capable of causing self-sustaining epidemics ($R_c > 1$) (red). (C) Density functions showing the likelihood of different values of TDR occurring. Dotted curves show density functions for the relative transmissibility for all of the strains with single-class resistance to NNRTIs (red; median 86%, IQR 81-89%) and dual-class resistance to NRTIs and NNRTIs (green; median 69%, IQR 60-76%) that are circulating in the current network in San Francisco. Solid curves show density functions for the relative transmissibility of NNRTI-resistant strains with $R_c > 1$; single-class NNRTIs (red; median 87%, IQR 83-90%) and dual-class NRTIs and NNRTIs (green; median 82%, IQR 78-86%). Transmissibility is defined relative to wild-type.

Fig. 4. A pruned version of the optimal tree. The root node contains data from the 3,827 filtered Monte Carlo simulations generated by the amplification cascade model; filtered simulations are after model calibration [Section 3 (9)]. Inside each node is the total number of simulations it contains, as well as the distribution of the response variable TDR. Low levels of TDR (<15%) are blue, while high levels of TDR (>15%) are red. The most important variable (IS=100) is $\nu^{-1}$, the average time (at the population level) it takes for CD4 cell counts in infected individuals to fall below 350 cells/microL. The variable $\alpha_1^W$ reflects the degree of infectivity of wild-type strains during the asymptomatic stage of infection, where infectivity is specified as the probability of transmitting HIV during one sex act. The variable $\alpha_1^T$ represents the probability that an individual receiving a current ARV regimen transmits HIV during one sex act. The remaining variables are: the transmissibility of strains (relative to wild-type) with single-class resistance to NRTIs ($\lambda_2$), the transmissibility of strains (relative to wild-type) with dual-class resistance to NRTIs and NNRTIs ($\lambda_5$), the average time spent in the treatment-eligible state (i.e., CD4 ≤ 350 cells/microL) by an untreated individual infected with a strain with single-class resistance to NRTI ($\mu_2^{-1}$), and the percentage of individuals with PI resistance who are virally suppressed ($\gamma_4$). The optimal tree has 84% predictive power in correctly identifying which simulations will generate high levels of TDR and 82% predictive power in correctly identifying which simulations will generate low levels of TDR.