

Antiretroviral Drugs for Tuberculosis Control in the Era of HIV/AIDS

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Human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) has dramatically increased the incidence of tuberculosis (TB) in sub-Saharan Africa, where up to 60% of TB patients are coinfecting with HIV and each year 200,000 TB deaths are attributable to HIV coinfection. Now HIV threatens control of TB in Asia, Eastern Europe, and Latin America. Antiretroviral (ARV) drugs can prevent TB by preserving immunity, but cohort analysis shows that early therapy, plus high levels of coverage and compliance, will be needed to avert a significant fraction of TB cases. However, ARV drugs could enhance the treatment of TB, and TB programs provide an important entry point for the treatment of HIV/AIDS.

In the decades leading up to 1980, tuberculosis (TB) was in decline throughout the world. There was reason to believe that if control efforts were maintained, and where necessary strengthened, TB would be driven steadily toward elimination. However, 30% of people in sub-Saharan Africa are latently infected with *Mycobacterium tuberculosis* (1), and the rapid spread of human immunodeficiency virus (HIV) during the 1980s and 1990s led to a similarly rapid increase in the incidence of TB, with notification rates in some countries increasing by more than five times in 10 years (2). HIV/acquired immunodeficiency syndrome (HIV/AIDS) control strategies have not substantially reduced the prevalence of HIV in sub-Saharan Africa (3), and the decline in immunity in people coinfecting with HIV and TB has meant that even good TB control programs based on short-course chemotherapy (4) have not been sufficient to contain the rising incidence of TB (5). As HIV spreads through Asia, Eastern Europe, and Latin America (3), TB control could be compromised there also. The development of new classes of antiretroviral (ARV) drugs (6), the availability of cheap generic equivalents (7), and the increasing commitment of international donors to making ARV drugs widely available in poor countries (8) should all help to reduce HIV-related illness and death over the next few years. Whether ARV drugs have a substantial impact on TB depends on their efficacy in preventing disease and prolonging life, on population coverage and patient compliance, and on synergies between the treatment of TB patients and the provision of ARV therapy to patients who are HIV-positive.

HIV infects and kills CD4⁺ T lymphocytes, and the concentration of CD4⁺ cells declines as

HIV infection progresses; this breakdown of immunity increases susceptibility to new *M. tuberculosis* infection and permits the reactivation of latent infection. ARV drugs block virus replication and reduce the plasma viral load to undetectable levels, allowing a degree of immune reconstitution. Here we bring together

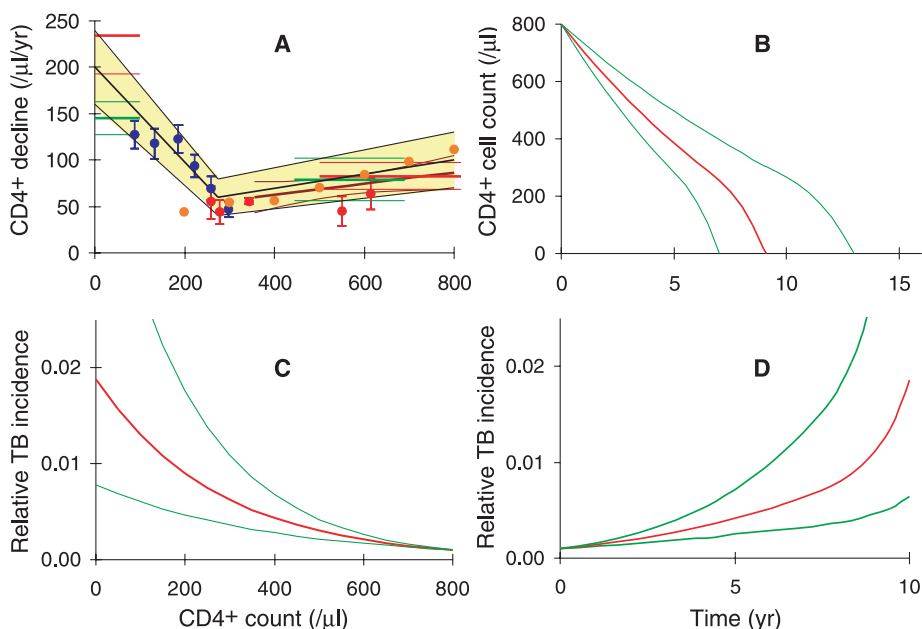


Fig. 1. Data used to estimate the incidence of TB in a cohort of people infected with HIV. (A) Estimated rates of decline of CD4⁺ count as a function of CD4⁺ count with 95% confidence limits. Data sources are as follows: brown lines (74); red lines (15); green lines (18); blue dots (16); orange dots (17); red dots, point estimates (9–13). The horizontal lines indicate results for studies in which the rate of decline was constant over the given range of CD4⁺ cell count. For the red and green lines running from 0 to 100 CD4⁺ cells/ μ l, time was measured to the onset of AIDS. These represent selected groups of rapid seroconverters and may be regarded as an estimate of the maximum rate of decline among people who have advanced HIV infection. The heavy black line in the middle gives the expected rate of decline of CD4⁺ count from the aggregate data; the yellow polygon indicates the range. (B) CD4⁺ count plotted against time for the expected rate of decline in (A) (red line) with confidence limits corresponding to the yellow polygon in (A) (green lines). (C) Estimated relative TB incidence as a function of CD4⁺ count (red line; confidence limits, green lines) for a population in which the CD4⁺ count, immediately after seroconversion, is 800/ μ l and the annual incidence of TB is 100 cases per 100,000 people per year. (D) Incidence of TB as a function of time estimated from (B) and (C), assuming a nominal incidence of TB immediately after seroconversion of 100 cases per 100,000 people per year.

data of four kinds, within a single analytical framework, in order to determine the potential population-level impact of ARV drugs on TB.

First, the rate of decline of CD4⁺ count in people infected with HIV has been determined at different levels (9–13) and over different ranges of CD4⁺ count (14–19). The form of the decline over time has been characterized as exponential (20), quadratic (19), and linear or piecewise linear (15, 18). The rates of decline of CD4⁺ count from 10 studies are shown in Fig. 1A [supporting online material (SOM) text]. The aggregated data suggest that as the CD4⁺ count declines from 800 to 300/ μ l, the rate of decline slows from about 100/ μ l/year to 60/ μ l/year. Below 300/ μ l, the rate of decline, measured over a few months, increases again to 150/ μ l/year as CD4⁺ count approaches 100/ μ l. At a very low CD4⁺ count, the rate of decline is between 150 and 250/ μ l/year. On the basis of these data, our best estimate of the decline of CD4⁺ count with time is as shown in Fig. 1B, which predicts a median survival time after infection with HIV of 9 years (range, 7 to 13 years), which is consistent with independent estimates of the median time to death from cohort studies (21, 22).

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Second, the incidence of culture-positive TB as a function of CD4⁺ count has been estimated in three studies, among which the incidence of TB varied by a factor of 60. For a reduction of 200 CD4⁺ cells/ μ l, the incidence of TB in these studies increased by factors of 1.6 (range, 1.0 to 2.5) (9), 2.4 (range, 1.2 to 4.7) (23), and 2.1 (range, 1.6 to 2.8) (24) (here and elsewhere, ranges are 95% confidence limits) (SOM text). It is also important to know whether the variation of TB incidence with CD4⁺ count depends on whether people are infected, uninfected, or anergic with regard to TB, as determined by skin testing with purified protein derivative (PPD). In a study in Italy (23), the incidence of TB increased by a factor of 3.2 (range, 1.3 to 8.1) when PPD-negative people were compared with anergic people and by about the same factor in a comparison of anergic and PPD-positive people. This was true across all levels of CD4⁺ count, and the interaction between PPD status and CD4⁺ count was not significant (SOM text). In a study of HIV-positive patients in the United States (25), the incidence of TB was 11.3 (range, 3.8 to 33.7) times higher in PPD-positive than in PPD-negative patients, which is in agreement with the Italian study. In sum, these data suggest that the incidence of TB increases by a factor of 2.1 (range, 1.4 to 3.0) for each reduction of 200/ μ l in the CD4⁺ count (weighted geometric mean) as shown in Fig. 1C, and that this increase is independent of PPD status or the background incidence of TB.

Combining the decline in CD4⁺ count over time (Fig. 1B) with the increase in TB incidence as a function of CD4⁺ count (Fig. 1C) gives TB incidence as a function of time since infection (Fig. 1D) (SOM text). We calculate that the median CD4⁺ count at which HIV-infected people develop TB is 256/ μ l (range, 207 to 310/ μ l), which is higher than, but not significantly different from, the median CD4⁺ count of 202/ μ l (range, 136 to 269/ μ l) observed in HIV-positive TB patients (SOM text). Because

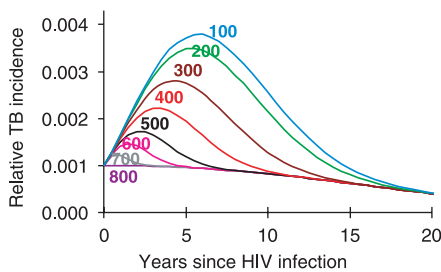


Fig. 2. Relative incidence of TB in a cohort of newly infected people as a function of time and of the CD4⁺ count below which people are given triple therapy (inset numbers indicate CD4⁺ cell count/ μ l). The nominal incidence of TB immediately after seroconversion is 100 cases per 100,000 people per year; if the background incidence were higher or lower, the curves would be scaled up or down accordingly.

the onset of severe pulmonary TB is known to be associated with a fall in CD4⁺ count (26), the difference is in the expected direction.

The third set of data describes the efficacy of ARV drugs in preventing TB. In a study in Cape Town, South Africa (24), ARV therapy for patients with CD4⁺ counts below 200/ μ l, between 200 and 350/ μ l, and above 350/ μ l reduced the incidence of TB in all three groups to that in the group with the highest CD4⁺ count (SOM text). This suggests that ARV therapy reduces the incidence of TB to the level observed soon after seroconversion.

The fourth piece of information is the survivorship of persons infected with HIV. In an earlier study, we found that without ARV therapy and for a mean age at seroconversion of 30 years, survivorship follows a Weibull distribution, with a median survival time of 10.2 ± 0.5 years. The Weibull shape parameter was 2.28 ± 0.12 , implying that the increase of mortality with time since infection is close to linear. With ARV therapy, the shape of the survivorship curve remains the same, but life expectancy increases to 19.8 ± 2.2 years (27).

These four groups of data can be combined to calculate the incidence of TB over time in a

cohort of HIV-infected patients, allowing the time at which ARV therapy is provided after HIV infection to vary (SOM text). Given the evidence that ARV therapy reduces the incidence of TB to the level observed immediately after HIV seroconversion (24), the risk of TB per person alive will be constant through time in cohorts that start ARV therapy immediately after HIV seroconversion (Fig. 2, line 8). The total number of TB cases then follows the Weibull survivorship curve, with a median of about 20 years. In cohorts that start ARV therapy at a lower CD4⁺ count, the number of TB cases initially increases while most people are still alive and their CD4⁺ count is falling, but declines once they start ARV therapy and declines further as they die (Fig. 2, lines 7 to 1). Without ARV therapy, and assuming a life expectancy of 10 years, the mean time between HIV infection and the development of TB is 6.0 years, consistent with the observed time lag of about 6 years between rising TB notifications and HIV incidence in East Africa (28). With ARV therapy, the mean time between HIV infection and the development of TB varies from 7.2 to 8.6 years, depending on the CD4⁺ count at which therapy is started.

Using the data in Fig. 2 we can determine the reduction in the incidence of TB over 20 years as a function of the CD4⁺ count at which ARV therapy is initiated and the effective coverage; that is, the product of population coverage and compliance (Fig. 3A). Current recommendations for developing countries are that ARV therapy should begin when the CD4⁺ count falls below 200/ μ l (29). With complete coverage and perfect compliance, this would reduce the cumulative incidence of TB in people infected with HIV by just 22% over 20 years; with an effective coverage of 50%, only 11% of TB cases would be prevented (Fig. 3A). To have a substantial impact on the incidence of TB in high-burden countries, it will therefore be necessary to start therapy early and to achieve high coverage and compliance. But even if ARV therapy begins at 500 CD4⁺ cells/ μ l, with 85% effective coverage TB incidence would be cut by only 50% over 20 years (Fig. 3A). The explanation for this low impact is that ARV drugs suppress TB but extend life expectancy, so HIV-infected individuals on treatment develop TB at a lower rate but over a longer period of time (Fig. 2). Because this is a cohort model it does not include the additional impact of ARV drugs on TB transmission. However, that additional impact is likely to be small, even in sub-Saharan Africa, because the proportion of TB transmission events from people dually infected with HIV is estimated to be less than 10% (1, 28).

Much of the TB arising in HIV-positive individuals is due to the breakdown of latent infections, so it should be possible to reduce TB incidence further by treating these latent infec-

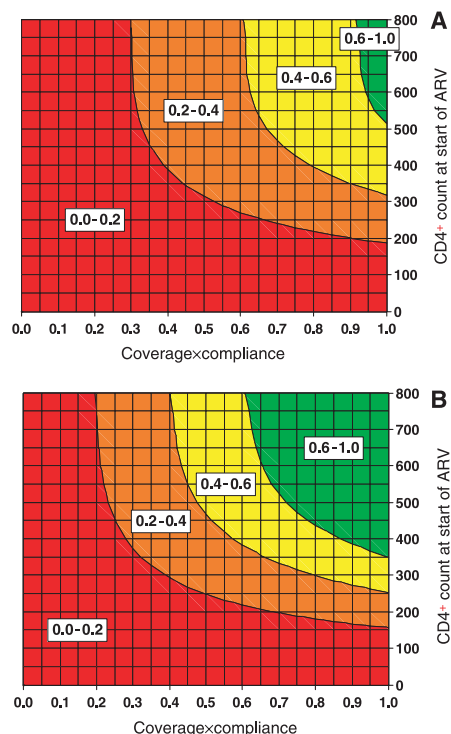


Fig. 3. Proportional reduction in the incidence of TB over 20 years among HIV-positive people (inset boxes) as a function of effective coverage (actual coverage \times compliance) and the CD4⁺ count/ μ l at which people start ARV therapy. (A) Assuming that the effect of ARV therapy is to reduce the incidence of TB to the level seen immediately after seroconversion. (B) As in (A), but assuming that TB preventive therapy is also given and that this reduces the incidence of TB in people on ARV therapy to zero.

tions; for example, with isoniazid as an adjunct to ARV therapy (30). Furthermore, there is evidence for a significant decline in CD4⁺ count and a corresponding increase in TB incidence soon after seroconversion (SOM text). If we assume, optimistically, that the joint effect of isoniazid and ARV drugs is to eliminate the risk of TB, the impact on the long-term transmission of TB would be greater (Fig. 3B). Under these circumstances, and assuming 85% coverage, starting ARV therapy at 500/ μ l would reduce the number of TB cases among those who are HIV-positive by 70%; starting at 200/ μ l would only reduce the number of cases by 25% (Fig. 3B).

The conditions under which ARV drugs could prevent a significant fraction of TB cases are therefore demanding, requiring treatment early in the course of HIV infection, plus high coverage and high compliance. As yet, no low- or middle-income country comes close to satisfying these requirements, with the possible exception of Brazil (31).

For countries that are attempting to manage interacting epidemics of TB and HIV/AIDS, one way to proceed would be to strengthen national TB control programs and use them as a point of entry for ARV therapy. A strong reason for doing so is that HIV infection is concentrated among TB patients; in parts of southern Africa, more than 60% of TB patients are HIV-positive, and in the World Health Organization African Region, 31% of TB cases and 40% of TB deaths are directly attributable to HIV (1). Furthermore, as shown above, TB patients who are infected with HIV generally present to health services with CD4⁺ counts in the range of 150 to 300/ μ l, the currently recommended level at which ARV therapy should start in nonindustrialized countries (29). Taking this approach, the immediate role for ARV drugs in TB control would be in supporting curative treatment and ensuring long-term survival, rather than as a mechanism of prevention. For an HIV-infected TB patient, neither anti-TB drugs nor ARV drugs alone will significantly extend life expectancy, but the two kinds of therapy should be powerful in combination.

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Supporting Online Material

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Resistance to Enediyne Antitumor Antibiotics by CalC Self-Sacrifice

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Antibiotic self-resistance mechanisms, which include drug elimination, drug modification, target modification, and drug sequestration, contribute substantially to the growing problem of antibiotic resistance among pathogenic bacteria. Enediynes are among the most potent naturally occurring antibiotics, yet the mechanism of resistance to these toxins has remained a mystery. We characterize an enediyne self-resistance protein that reveals a self-sacrificing paradigm for resistance to highly reactive antibiotics, and thus another opportunity for nonpathogenic or pathogenic bacteria to evade extremely potent small molecules.

Antibiotic production within actinomycetes represents a prominent instrument of natural self-preservation. These toxic secondary metabolites—small, highly functionalized molecules—are produced and often secreted, creating a local protective environment that is inhospitable to invading organisms. Because antibiotics often function by disrupting basic biological processes (e.g., DNA, protein, or cell-wall biosynthesis), antibiotic-producing organisms have evolved numerous mechanisms to evade their own chemical warfare (1). These self-resistance mechanisms are often shared among bacteria and have contributed substantially to the serious problem of antibiotic resistance among pathogenic bac-

teria (2). Within the repertoire of naturally occurring antibiotics, the enediynes such as calicheamicin γ_1^I (1) (Fig. 1) are examples of nature's ingenuity in both their architecture and effectiveness as DNA-cleaving agents. Since the susceptibility of some organisms to 1 is in the femtomolar range (3), how does an organism that produces such a potent molecule thrive? Recently, the locus of encoding for the calicheamicin (*cal*) biosynthesis was elucidated (4), which relied in part upon clones that carry a gene (*calC*) conferring resistance to 1 in vivo (5). The role of calicheamicin as a "chemical nuclease" has been extensively studied (6), and although *calC* was shown to convey intracellular 1 resistance in *Escherichia coli* (5), its mechanism of action has remained undefined.

We created a wild-type *calC* construct within *E. coli* BL21(DE3), designated pJB2011-*E. coli*, for the efficient production and purification of the encoded CalC protein (7). Previous agarose gel-based assays revealed CalC to be involved in the inhibition of 1-dependent double-stranded DNA (dsDNA) scission, as evident by the qualita-

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