Appendix B: DESCRIPTION OF HIV PROGRESSION SIMULATION *

Our simulation separately tracks the number of accumulated genetic mutations that may confer resistance to each of the three main drug categories of HAART: Nucleoside Reverse Transcriptase Inhibitors (NRTIs), Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs), and Protease Inhibitors (PIs). It then uses this information to determine the likelihood of phenotypic resistance to HAART. Mutations develop as a direct function of the viral replication rate and the mutation rate; whether these mutations persist in the viral population is related to whether there is selection pressure from the presence of a particular medication in the patient's regimen. Phenotypic resistance and adherence determine the likelihood of HAART effectiveness, which then impacts the likelihood of clinical outcomes. Suppression of viral load impacts the CD4 count trajectory favorably, which in turn reduces HIV-related morbidity and mortality.

Numerous longitudinal studies have described the incidence of individual mutations in the HIV reverse transcriptase and protease genes and their correlation with phenotypic resistance and clinical characteristics.[1-30] While their results were heterogeneous, several principles emerged. First, mutations accrue in response to selection pressures based on drugs in the antiretroviral regimen. For example, if the HAART round includes NNRTIs but not PIs, a mutation conferring resistance to an NNRTI is far more likely to accrue than is a mutation conferring resistance to a PI. Second, adherence is an important modulator of selection pressures. When adherence is low, selection pressures will decrease, so even though a high rate of viral replication may potentially give rise to a resistant mutation, this impact is mitigated as there is no selection pressure to sustain the mutation in the viral population. Third, the rate of accruing mutations depends on the number of drugs to which HIV is susceptible. The rate is lower if the number of susceptible drugs is high. Fourth, the rate of accruing mutations is unlikely to be zero, even if the round includes three or more drugs to which there is complete susceptibility of HIV and therefore maximum suppression of viral replication. Fifth, if there is resistance to all drugs in the HAART regimen, selection pressure for additional mutations will be low, and it is unlikely that additional mutations will accrue. (For this reason, even though the model does not specify a "ceiling" on the number of mutations, after patients have accumulated enough mutations to accrue resistance to all drugs in a class; it is unlikely that they will accumulate additional mutations within that class). Lastly, the cumulative incidence of resistance mutations is more clinically important than their point prevalence because

accumulated resistance mutations are usually archived, and therefore may influence the effectiveness of HAART regimens even if they no longer are detected by assay. (Therefore, the simulation should represent the cumulative incidence of mutations, rather than the point prevalence.)

After these basic principles were used to specify the model, we calibrated it using clinical data from a large observational cohort study until time to treatment failure and survival replicated clinical observations in a large observational study.[31] We now describe in greater detail the simulation's specification of genotypic mutations, phenotypic resistance, viral load trajectories, CD4 trajectories, and mortality.

Genotypic Mutations

A theoretical construct entitled *optimal mutation accumulation rate* is the starting point for determining all mutation accumulation rates in the model. This construct denotes what the mutation accumulation rate would be under optimal circumstances (perfect adherence to therapy and no resistance to therapy). A different *optimal mutation accumulation rate* may be specified for each drug class; however, during our calibration of the model, we found that it was not necessary to do this in order to yield clinically accurate projections of time to treatment failure and survival.

Starting from the *optimal mutation accumulation rate*, the model calculates the *actual mutation accumulation rate* based on the amount of viral replication (proxied by viral load) and the level of adherence:

Actual mutation accumulation rate = *optimal mutation rate* * (*replication factor* ^ (log *instantaneous viral load* – 2.31)) * (*adjustment factor for composition of regimen*)* (*adherence adjuster*)

Replication factor was set at 3.16 based on results from heterogeneous studies that measured mutation accumulation rates with varying viral loads.[4,7-9,20,29,30] Note that the mutation rate increases as viral load (and viral replication) increases. It was not necessary to change this estimate during model calibration.

Adherence adjuster decreases the mutation accumulation rate based on the amount of nonadherence. This was specified so as to be logically consistent with other assumptions embedded in the model (e.g. if an individual is completely non-adherent to a particular drug, the impact on the accumulation of same-class mutations should be the same as if the person was not prescribed the drug at all).

Adjustment factor for composition of regimen ensures that mutations only accumulate to the drug types that are represented in the current HAART round (e.g. if an NNRTI is not included in the current HAART round, it is extremely unlikely to get an NNRTI mutation).

During calibration of our model, we found that an *optimal mutation rate* of 0.014 per month yielded the closest correlation of observed versus expected results for time to treatment failure and survival, and that any mutation rate between 0.010 per month and 0.015 per month yielded reasonably good correlations. Note that this rate reflects mutation accumulation under optimal circumstances (perfect adherence and no resistance), and therefore individuals in the model, on average, accumulate mutations more rapidly.

Phenotypic Resistance

The model considers the possibility that any one particular mutation may not induce any resistance, may engender resistance to one drug, or may engender resistance to more than one drug (because of cross-resistance). Estimates for cross resistance in Table 2 were based on published sources that specify the relationship of each individual mutation with each HAART drug,[32] and incorporates a mathematical average of how likely any one mutation is likely to engender resistance to more than one drug in the same class. Because a separate calculation is performed for each drug class, the model captures clinically observed heterogeneity among drug classes (i.e. it is more common among NNRTIs than among PIs or NRTIs).

The model also considers the possibility that a particular mutation may or may not result in phenotypic resistance. Because this likelihood varies greatly by individual mutation, we used a simple summary estimate (0.5) which fell within the clinically observed range (approximately 0.1 to 0.9).[4,8,12,16,21,22,29,30] Since the rate of accumulating resistance in the model is the product of the rate of accumulating mutations and the probability

that each mutation will cause resistance, any error in this estimate would have induced a compensatory error in the imputed mutation rate, and therefore would not have been expected to adversely impact its results. Because the imputed mutation rate was remarkably consistent with clinical observations, it is unlikely that this error was substantial.

Change in Viral Load

Our model assumes that the viral load for each patient has a "set point" that reflects the particular dynamics between the virulence of the HIV strain and the activity of the immune system. In the current analyses, we assume that the viral load prior to starting HAART reflects this "set-point." (Therefore the current analyses will not apply to primary HIV infection, which is characterized by very high viral loads that are transient.) The model assumes that the viral load decreases after HAART is started and that the extent of the decrease varies with the number of drugs in the HAART round to which there is phenotypic resistance and with the degree to which the patient adheres to the HAART round. If mutations accrue and resistance develops, the viral load will start to increase and move toward its set point. Similarly, if a patient stops taking one or more drugs, the viral load will start to move toward its set point, with the speed of movement depending on the number of drugs and doses missed.

We distinguish between *steady state viral load* (a theoretical, immeasurable construct) and *instantaneous viral load* (a measurable construct). *Steady state viral load* is the viral load that would be reached at equilibrium, after an infinite amount of time, if there were no changes in any of its determinates. *Instantaneous viral load* is the true viral load at a particular time. We distinguish between these constructs because the determinates of viral load may change by clinically significant amounts over much shorter time scales (i.e. over hours) than the true viral load (i.e. usually over weeks or months). The *instantaneous viral load* moves towards the *steady state viral load*, with a delay factor that reflects its slower kinetics.

Steady-state viral load

Based on our previously reported analyses of antiretroviral naïve individuals in care,[31] *steady state viral load* is determined by the following equations:

Log *steady state viral load* = Log *viral load "set point"* – Log *viral load decrement*

Log *Viral load decrement* = (Log *viral load "set point"* * 0.891 – 1.6) * (*adherence adjuster*) * (*resistance adjuster*)

Here, the *viral "set point"* is the equilibrium viral load after the primary phase of HIV infection has concluded.

Adherence adjuster attenuates the decrease in viral load as individuals are more non-adherent to therapy. Adherence is defined as the proportion of antiretroviral doses taken as directed. We assume a linear relationship between adherence and the logarithm of the decrease in viral load, a reasonable approximation as verified by a later analyses of 6,394 antiretroviral naïve patients for which adherence information was available (Veterans Aging Cohort Study; unpublished data).

Resistance adjuster attenuates the decrease in viral load if patients have resistance to one or more antiretroviral drugs. We assume a linear relationship between the proportion of drugs to which there is resistance, and the logarithm of the decrease in viral load (i.e. if viral load decrease would be X log units with resistance to no drugs, it would be 2/3 * X log units with resistance to 1 drugs, and 1/3 * X log units with resistance to 2 drugs). While this is clearly a simplification, there is a growing literature to support a rough rule of thumb that, under favorable circumstances (the absence of resistance and high levels of adherence), one-drug regimens drop log viral loads by approximately 1 log, two drug regimens drop log viral loads by approximately 2 logs, and three drug regimens drop log viral loads by approximately 3 logs.[7,9,11,13,18,20,29] Over time, we may be able to make this relationship more precise.

Instantaneous viral load

The formula for this variable is straightforward, specifying an exponential convergence towards the *steady state viral load*:

Log *Instantaneous viral load* at time t = Log *Instantaneous viral load* at time t-1 + (Log *instantaneous viral load* at time t - Log *instantaneous viral load* at time t-1)/*viral load delay constant.*

Viral load delay constant was set at 1.5 months, reflecting observed kinetics of viral load fluctuations.

Change in CD4 Count

The CD4 count plays a crucial role in determining the risk of HIV-related mortality, and therefore estimating its trajectory is essential for predicting this mortality risk over long time periods.
Similarly to how viral load is represented, CD4 count is also represented by a steady state variable and an instantaneous variable.

Steady State

The representation of CD4 is more complicated than viral load because there is no "set point." Published data prior to widespread adoption of HAART suggests that the CD4 count declines at a rate inversely proportional to the viral load.[33] However, HAART may change this relationship substantially. We therefore analyzed the CD4 count trajectories of the anti-retroviral naive HIV-positive patients starting HAART in the same observational cohort that was used to analyze viral load. Using statistical models that controlled for important covariates, we found that changes in CD4 count during HAART may be disaggregated into two separate components: a *trough to peak* change in CD4 (representing the rise in CD4 count from when a round is started, to the highest level that will be obtained during that round) and a *trough to trough* change in CD4 (representing the change in CD4 count from the start of first HAART round to the start of each subsequent round). The "peak" CD4 for a particular HAART round was approximated by the value 1 year after that round was initiated. (This is only a

gross approximation, as data show that CD4 counts continue to increase as long as regimens are effective. However, the rate of increase diminishes dramatically after 1 year, and therefore the 1 year value can be used as a proxy for the plateau.)

If off HAART

CD4 at time t = *CD4 at time t-1* –time interval (in months) * (1.78+2.8*(log *instantaneous viral load* – 3).

If on HAART

CD4 = CD4 count at start of HAART + *trough to peak change* + *trough to trough change.*

Trough to peak change = (105 + 24*(log *viral load "set point"* – log *instantaneous viral load*) -80 (if on second HAART round) -69 (if on third HAART round) – 76 (if on fourth HAART round) – 143 (if after fourth HAART round)) * *adherence adjuster*.

Trough to trough change (from start of first round to start of later round) = 61 (if starting second HAART round) + 24 (if starting third HAART round) + (11 (if starting fourth HAART round) -62 (if starting fifth HAART round – 93 (if starting sixth or greater HAART round).

Because *trough to peak change* should be always be positive, and in rare circumstances this expression may produce a negative result (with later HAART rounds that are ineffective), additional programming is used to limit the lower bound of this expression at 0.

Instantaneous

Instantaneous CD4 incorporates a delay factor (3 months), analogous to *instantaneous viral load*. It also incorporates a "noise" factor to reflect unexplained variance in the CD4 count (a far lower proportion of CD4

count variance is explained by covariates). The delay factor was specified based on observed CD4 kinetics, and the noise factor was specified during the verification of the model to result that clinically plausible CD4 trajectories were produced for simulated individual patients.

Adherence

The model permits the user to specify an overall predisposition towards adherence to therapy, defined as the proportion of antiretroviral doses taken as directed. Because adherence often varies greatly from time to time (both within regimens, and because of differing regimen characteristics, between regimens), we modify this predisposition via two "noise" terms, one of which is drawn anew with each time period, and the other of which is drawn anew each time a new regimen starts. The noise factors were specified during the verification of the model to result in clinically plausible viral load and CD4 trajectories for individual patients.

The model incorporates the observation that nonadherence to one drug in a HAART regimen is often highly correlated with nonadherence to other HAART drugs in the regimen (i.e. if you miss one drug, chances are relatively high that you also will miss the other drugs at the same dosing time). To represent this correlation, the model permits the user to select a correlation factor. Because there are insufficient clinical data on which to base estimates for this correlation, we arbitrarily set it at 0.9 for all of our analyses. This setting was consistent with satisfactory performance on our calibration and validation exercises.[31]

Mortality

Mortality is partitioned into *HIV-related* and *non-HIV-related* sources of death. *HIV-related mortality* is a function of age, CD4 count, viral load, and presence of HAART (to consider the salutary effects of maintaining less "fit" viral strains, independent from its beneficial impact on CD4 count and viral load). Estimates were based on our analyses of observational data of HIV+ individuals for which cause of death was a prospectively

defined and measured outcome. Detailed tables are available from the authors on request; summary tables were published previously.[31]

Non-HIV related mortality is a function of age, sex, and race, and was based on same observational cohort above. These results were indexed to published life tables of all-causes mortality in the United States by age, race, and sex to extrapolate beyond the age groups that were represented in this population.[34] Detailed tables are available from the authors on request; summary tables were published previously.[31]

*Adapted from online appendix in Braithwaite RS, Shechter S, Roberts MS, et al. Explaining variability in the relationship between antiretroviral adherence and HIV mutation accumulation. J Antimicrob Chemother. 2006; 58(5):1036-1043.

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