Predicting the outcomes of treatment to eradicate the latent reservoir for HIV-1

The MIT Faculty has made this article openly available. Please share how this access benefits you. Your story matters.
Predicting the outcomes of treatment to eradicate the latent reservoir for HIV-1

Alison L. Hill*a,b,1, Daniel I. S. Rosenbloom*a,c,1, Feng Fu*d, Martin A. Nowak*, and Robert F. Siliciano*a,f

*Program for Evolutionary Dynamics, Department of Mathematics, and Department of Organismic and Evolutionary Biology, and aBiophysics Program and Harvard-MIT Division of Health Sciences and Technology, Harvard University, Cambridge, MA 02138; bDepartment of Biomedical Informatics, Columbia University Medical Center, New York, NY 10032; cInstitute of Integrative Biology, Eidgenössische Technische Hochschule Zürich, 8092 Zurich, Switzerland; and dDepartment of Medicine and Howard Hughes Medical Institute, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Edited by John M. Coffin, Tufts University School of Medicine, Boston, MA, and approved July 9, 2014 (received for review April 12, 2014)

Massive research efforts are now underway to develop a cure for HIV infection, allowing patients to discontinue lifelong combination antiretroviral therapy (ART). New latency-reversing agents (LRAs) may be able to purge the persistent reservoir of latent virus in resting memory CD4+ T cells, but the degree of reservoir reduction needed for cure remains unknown. Here we use a stochastic model of infection dynamics to estimate the efficacy of LRA needed to prevent viral rebound after ART interruption. We incorporate clinical data to estimate population-level parameter distributions and outcomes. Our findings suggest that ~2,000-fold reductions are required to permit a majority of patients to interrupt ART for 1 y without rebound and that rebound may occur suddenly after multiple years. Greater than 10,000-fold reductions may be required to prevent rebound altogether. Our results predict large variation in rebound times following LRA therapy, which will complicate clinical management. This model provides benchmarks for moving LRAs from the laboratory to the clinic and can aid in the design and interpretation of clinical trials. These results also apply to other interventions to reduce the latent reservoir and can explain the observed return of viremia after months of apparent cure in recent bone marrow transplant recipients and an immediately-treated neonate.

The latent reservoir (LR) for HIV-1 is a population of long-lived resting memory CD4+ T cells with integrated HIV-1 DNA (1). After establishment during acute infection (2), it increases to 107 to 108 cells and then remains stable. As only replicating virus is targeted by antiretroviral therapy (ART), latently infected cells persist even after years of effective treatment (3, 4). Cellular activation leads to virus production and, if treatment is interrupted, viremia rebounds within weeks (5). Several molecular mechanisms maintain latency, including epigenetic modifications, transcriptional interference from host genes, and the absence of activated transcription factors (6–9).

Major efforts are underway to identify pharmacologic agents that reverse latency by triggering the expression of HIV-1 genes, and the absence of activated transcription factors (6–9). This approach has been particularly useful in understanding HIV-1 treatment. Previous models have explained the multiphasic decay of viremia (30), the limited inflow to the LR during treatment (31), the dynamics of viral blips (32), and the contributions of the LR to drug resistance (33). No model has yet been offered to describe the effect of LRAs. Here we present a novel modeling framework to predict the degree of reservoir reduction needed to prevent viral rebound following ART interruption. The model can be used to estimate the probability that cure is achieved, or, barring that outcome, to estimate the length of time following treatment interruption before viral rebound occurs (Fig. 1A).

Results

Determination of Key Viral Dynamic Parameters Governing Patient Outcomes. We use a stochastic model of HIV-1 reservoir dynamics and rebound that, in its simplest form, tracks two cell types: productively infected activated CD4+ T cells and latently infected resting CD4+ T cells (Fig. 1B). A latently infected cell can either activate or die, each with a particular rate constant. An actively infected cell can produce virions, resulting in the

Significance

HIV infection cannot be cured by current antiretroviral drugs, due to the presence of long-lived latently infected cells. New antilatency drugs are being tested in clinical trials, but major unknowns remain. It is unclear how much latent virus must be eliminated for a cure, which remains difficult to answer empirically due to few case studies and limited sensitivity of viral reservoir assays. In this paper, we introduce a mathematical model of HIV dynamics to calculate the likelihood and timing of viral rebound following antilatency treatment. We derive predictions for the required efficacy of antilatency drugs, and demonstrate that rebound times may be highly variable and occur after years of remission. These results will aid in designing and interpreting HIV cure studies.


The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

1A.L.H. and D.I.S.R. contributed equally to this work.

2To whom correspondence should be addressed. Email: ahill@fas.harvard.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1406663111/-/DCSupplemental.

www.pnas.org/cgi/doi/10.1073/pnas.1406663111

PNAS Early Edition | 1 of 6
active infection of some number of other cells, or it can die from other causes without producing virions that infect other cells. In the latter case, cytotoxic T lymphocyte (CTL) killing, errors in viral reverse transcription, or other problems upstream of virion production may prevent further infection. The model only tracks the initial stages of viral rebound, when target cells are not yet limited. A full description of the model is provided in Materials and Methods and SI Materials and Methods.

The initial conditions for the dynamic model depend on the number of latently infected cells left after LRA therapy. LRA efficacy is defined by the fraction \( q \) of the LR that remains following treatment. The model tracks each latent and active cell to determine whether viral rebound occurs, and if so, how long it takes. Importantly, no single activated cell is guaranteed to reestablish the infection, as it may die before infecting other cells. Even if it does infect others, those cells likewise may die before completing further infection. This possibility is a general property of stochastic models, and the specific value for the establishment probability depends on the rates at which infection and death events occur. Our goal is to calculate the probability that at least one of the infected cells remaining after therapy escapes extinction and causes viral rebound, and if so, how long it takes. If all cells die, then rebound never occurs and a cure is achieved. As the model only describes events after completion of LRA therapy, our results are independent of the therapy protocol or mechanism of action.

Using both stochastic simulations and theoretical analysis of this model, we find that the probability and timing of rebound are measured on the order of a few days, that so few latently infected cells survive that none reactivate and start a resurgent infection during the patient’s lifespan. In this case, LRA has essentially cleared the infection and cure is achieved. We simulated the model to predict the relationship between LRA efficacy and median time until rebound among patients who do not clear the infection (Fig. 2B). Roughly a 2,000-fold reduction in LR size is needed for median rebound times of 1 y. Only modest (about 2-fold) increases in efficacy and clearance (Fig. 2A). We find that the LR must be reduced 10,000-fold before half of patients are predicted to clear the infection.

If LRA therapy fails to clear the infection, the next-best outcome is extension of the time until rebound, defined as plasma HIV-1 RNA > 200 copies per mL. We computed the relationship between LRA efficacy and median time until rebound among patients who do not clear the infection (Fig. 2B). Roughly a 2,000-fold reduction in LR size is needed for median rebound times of 1 y. Only modest (about 2-fold) increases in median rebound time are predicted for up to 100-fold reductions in LR size. In this range, the rebound time is independent of latent cell lifespan (decay rate \( \delta \)) and is driven mainly by the reactivation rate \( (A) \) and the infection growth rate \( (r) \). The curve inflects upward (on a log scale) at ~100-fold reduction and eventually reaches a ceiling as
clearance of the infection becomes the dominant outcome. The upward inflection results from a change in the forces governing viral dynamics. If the reservoir is large (little reduction), then cells activate frequently, and the dominant component of rebound time is the time that it takes for viruses from the many available activated cells to grow exponentially to rebound levels; the system is in a growth-limited regime. If the reservoir is small (large reduction), the dominant component is instead the expected waiting time until activation of the first cell fated to establish a rebounding lineage; the system is in an activation-limited regime. Because waiting time is roughly exponentially distributed, times to rebound in this regime can vary widely among patients on the same therapy, even with identical values of the underlying parameters.

Survival curves, plotting the fraction of simulated patients maintaining virologic suppression over time, demonstrate the extreme interpatient variability and long follow-up times required for LRA therapy (Fig. 2C). For less than 100-fold reductions in LR size, simulated patients uniformly rebound within a few months because rebound dynamics are not in the activation-limited regime. If therapy decreases LR size 1,000-fold, then ~55% of patients are predicted to delay rebound for at least 6 mo. However, of these patients, 47% suffer rebound in the following 6 mo. Higher reservoir reductions lead to clearance in many patients. In others, rebound may still occur after years of apparent cure, posing a challenge for patient management.

Earlier work suggested a shorter reservoir half-life of 6 mo (35), indicating that dramatic decreases in LR size would occur after ~5 y or more of suppressive ART even in the absence of LRA therapy. We consider the prospects for HIV eradication or long treatment interruptions with this faster decay rate. In this optimistic scenario, only 1,500-fold reductions are needed for half of patients to clear the LR, and rebound becomes highly unlikely after a few years. Alternatively, in a worst-case scenario where latent cell death is perfectly balanced by homeostatic proliferation such that the reservoir does not decay at all (~0), much higher efficiencies are needed to achieve beneficial patient outcomes (Fig. 3).

Setting Treatment Goals with Uncertainty Considerations. We conducted a full uncertainty analysis of the model, by simultaneously varying all parameters over their entire ranges (Table 1 and Fig. S1). For each simulated patient, values for the three parameters \( \delta, A, \) and \( r \) were sampled independently from their respective distributions, whereas \( P_{\text{Est}} \) was sampled from a conditional distribution that depends on \( r \) (Materials and Methods). Results for this simulated cohort are similar to those for the point estimates, with greater interpatient variation in outcomes (Fig. 3A). This variation makes the survival curves less steep: Cure is slightly more likely at low efficacy, but slightly less likely at high efficacy. As expected from Eq. 1, cure is more likely for patients with lower \( A \) or \( P_{\text{Est}} \) values and higher \( \delta \) values. If therapy provides only 10 to 100-fold LR reductions, a subset of patients may delay rebound for several months.

Using these cohort-level predictions, we can set efficacy goals for the reservoir reduction needed to achieve a particular likelihood of a desired patient outcome. Fig. 4 provides target LRA efficacies for which 50% of patients are predicted to remain rebound-free for a specified interruption time. Reductions of under 10-fold afford patients only a few weeks to a month off treatment without rebound. For 1-y interruptions, a 1,000–3,000-fold reduction is needed. To achieve the goal of eradication (cure) a 4-log reduction is required. This value increases to 4.8 logs to cure 75% of patients, and to 5.8 logs for 95% of patients.

Table 1. Estimated values for the key parameters of the stochastic viral dynamics model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Estimation method</th>
<th>Ref(s)</th>
<th>Best estimate</th>
<th>Distribution*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LR decay rate</td>
<td>( \delta )</td>
<td>Long-term ART, ( \delta = \ln(2)/(1/12) )</td>
<td>(6, 7)</td>
<td>( 5.2 \times 10^{-4} ) d(^{-1} )</td>
<td>( \delta \sim \mathcal{N}(5.2, 1.6) \times 10^{-4} ) d(^{-1} )</td>
</tr>
<tr>
<td>LR exit rate</td>
<td>( A )</td>
<td>Viral rebound after ART interruption</td>
<td>(8, 69)</td>
<td>57 cells per day ( \log_{10}(A) \approx \mathcal{N}(1.76, 1.0) )</td>
<td></td>
</tr>
<tr>
<td>Growth rate</td>
<td>( r )</td>
<td></td>
<td></td>
<td>0.4 d(^{-1} ) ( \log_{10}(r) \approx \mathcal{N}(0.40, 0.19) )</td>
<td></td>
</tr>
<tr>
<td>Establishment probability</td>
<td>( P_{\text{Est}} )</td>
<td>Population genetic modeling</td>
<td>(71, 72)</td>
<td>0.069 ( \mathcal{C} )</td>
<td></td>
</tr>
</tbody>
</table>

*Notation \( X \sim \mathcal{N}(\mu, \sigma) \) means that \( X \) is a random variable drawn from a normal distribution with mean \( \mu \) and SD \( \sigma \).

Fig. 2. Clearance probabilities and rebound times following LRA therapy predicted from the model using point estimates for the parameters (Table 1). LRA log-efficacy is the number of orders of magnitude by which the LR size is reduced following LRA therapy, \( -\log_{10}(q) \). (A) Probability that the LR is cleared by LRA. Clearance occurs if all cells in the LR die before a reactivating lineage leads to viral rebound. (B) Median viral rebound times (logarithmic scale) among patients who do not clear the infection. (C) Survival curves (Kaplan–Meier plots) show the percentage of patients who have not yet experienced viral rebound, plotted as a function of the time (logarithmic scale) after treatment interruption. Solid lines represent simulations, and circles represent approximations from the branching process calculation. All simulations included \( 10^3 \) to \( 10^6 \) patients.
with current knowledge. When survival curves for larger cohorts become available, Bayesian methods can be used to update estimates in Table 1 and reduce uncertainty of future predictions.

Discussion

Our model is, to our knowledge, the first to quantify the required efficacy of LRAs for HIV-1 and set goals for therapy. For a wide range of parameters, we find that therapies must reduce the LR by at least 2 orders of magnitude to meaningfully increase time to rebound after ART interruption (upward inflection in Figs. 2B and 3A, II, B, II, C, II), and that reductions of approximately 4 orders of magnitude are needed for half of patients to clear the infection (Figs. 3A, I; B, I; C, I, and 4). Standard deviations in rebound times of many months are expected, owing to substantial variation in reactivation times after effective LRA therapy brings the infection to an activation-limited regime. Though the efficacy required for these beneficial outcomes likely exceeds the reach of current drugs, our results permit some optimism: We show for the first time, to our knowledge, that reactivation of all cells in the reservoir is not necessary for cessation of ART. This is because some cells in the LR will die before reactivating or, following activation, will fail to produce a chain of infections leading to rebound. On a more cautionary note, the wide distribution in reactivation times necessitates careful monitoring of patients, as rebound may occur even after long periods of viral suppression.

Even without any reservoir reduction, variation in infection parameters and chance activation together predict delays in rebound of at least 2 mo in a small minority of patients (Figs. 3A, III and 4), consistent with ART interruption trials such as SPARTAC (40). More detailed (and possibly more speculative) models including immune responses may be needed to explain midyear posttreatment control, such as seen in the VISCONTI cohort (24).

Our analysis characterizing the required efficacy of LRA therapy does not rely on the specific mechanism of action of these drugs, only the amount by which they reduce the reservoir. We have assumed that, after ART/LRA therapy ends, cell activation and death rates return to baseline. We have also assumed that the reservoir is a homogeneous population with constant activation and death rates. The presence of reservoir compartments with different levels of LRA penetration does not alter our results, as they are stated in terms of total reservoir residence. If, however, these compartments vary in activation or death rates, the presence of reservoir compartments with different levels of LRA penetration does not alter our results, as they are stated in terms of total reservoir residence. If, however, these compartments vary in activation or death rates (41), or if dynamics of activated cells depend on their location, fluorescent imaging studies of adenovirus have shown that a large majority of in vitro infections seeded by single productively infected cells die out early, before rapid growth and plaque formation can occur (54). Keeping other parameters constant, assuming a worst-case (highest) value for the establishment probability raises the reservoir reductions required for cure or a desired extended rebound time by 0.8 logs. Regardless of the exact probability, the stochastic nature of HIV-1 activation and infection dynamics implies that even similarly situated patients may experience divergent responses to LRA.

The model can also advise aspects of trial design for LRAs. Survival curves computed from [S8] can be used to predict the probability that a patient is cured, given that they have been off therapy for at least 2 mo in a small minority of patients (Figs. 3A, III and 4), consistent with ART interruption trials such as SPARTAC (40). More detailed (and possibly more speculative) models including immune responses may be needed to explain midyear posttreatment control, such as seen in the VISCONTI cohort (24).

Our analysis characterizing the required efficacy of LRA therapy does not rely on the specific mechanism of action of these drugs, only the amount by which they reduce the reservoir. We have assumed that, after ART/LRA therapy ends, cell activation and death rates return to baseline. We have also assumed that the reservoir is a homogeneous population with constant activation and death rates. The presence of reservoir compartments with different levels of LRA penetration does not alter our results, as they are stated in terms of total reservoir residence. If, however, these compartments vary in activation or death rates (41), or if dynamics of activated cells depend on their location, fluorescent imaging studies of adenovirus have shown that a large majority of in vitro infections seeded by single productively infected cells die out early, before rapid growth and plaque formation can occur (54). Keeping other parameters constant, assuming a worst-case (highest) value for the establishment probability raises the reservoir reductions required for cure or a desired extended rebound time by 0.8 logs. Regardless of the exact probability, the stochastic nature of HIV-1 activation and infection dynamics implies that even similarly situated patients may experience divergent responses to LRA.

The model can also advise aspects of trial design for LRAs. Survival curves computed from [S8] can be used to predict the probability that a patient is cured, given that they have been off therapy for at least 2 mo in a small minority of patients (Figs. 3A, III and 4), consistent with ART interruption trials such as SPARTAC (40). More detailed (and possibly more speculative) models including immune responses may be needed to explain midyear posttreatment control, such as seen in the VISCONTI cohort (24).

![Fig. 3. Predicted LRA therapy outcomes, accounting for uncertainty in patient parameter values. (A) Full uncertainty analysis where all viral dynamics parameters are sampled for each patient from the distributions provided in Table 1. (B) A best-case scenario where the reservoir half-life is only 6 mo ($\delta = 3.8 \times 10^{-3} \text{d}^{-1}$). All patients have the same underlying viral dynamic parameters, otherwise given by the point estimates in Table 1. (C) A worst-case scenario where the reservoir does not decay because cell death is balanced by homeostatic proliferation ($\delta = 0$). (I) Probability that the LR is cleared by LRA. Clearance occurs if all cells in the LR die before a reactivating lineage leads to viral rebound. LRA log-efficacy is the number of orders of magnitude by which the LR size is reduced following LRA therapy, $-\log_{10}(q)$. (II) Median viral rebound times (logarithmic scale) among patients who do not clear the infection. (III) Survival curves (Kaplan–Meier plots) show the percentage of patients who have not yet experienced viral rebound, plotted as a function of the time (logarithmic scale) after treatment interruption. All simulations included $10^6$ to $10^9$ patients.](image-url)
In this notation, $Y$ and $Z$ represent individual actively or latently infected cells, respectively, $\Theta$ represents no cells, and the arrows represent one type of cell becoming the other type. A latently infected cell can either activate (at rate $a$) or die (at rate $d_a$). An actively infected cell can either die (at rate $d$) or produce a collection of virions (at rate $b$) that results in the infection of $c$ other cells, where $c$ is a Poisson-distributed random variable with parameter $\lambda$. $p_i(c)$ represents the distribution $\left(\sum_{i=0}^{\infty} \frac{\lambda^i e^{-\lambda}}{i!}\right)$. After an infection event, the original cell dies.

Each event occurs independently within a large, constant target cell population. As the model does not include limitations on viral growth, it describes only the initial stages of viral rebound. Because clinical rebound thresholds (plasma HIV RNA $>50$–200 copies per mL) are well below typical set points ($10^4$ to $10^5$ copies per mL), this model simplifies to analyze rebound following LRA therapy and ART interruption. We do not explicitly track free virus, but assume it to be proportional to the number of infected cells. This assumption is valid because rates governing production and clearance of free virus greatly exceed other rates, allowing a separation of time scales. As we are not interested in blips or other intradyral viral dynamics, this assumption does not influence our results. A method for calculating the proportionality between free virus and infected cells is provided in SI Materials and Methods.

The growth rate of the infection is $r = b/(1 - 1 - d)$. The total death rate of infected cells is $d = b + a$, and the basic reproductive ratio (mean offspring number for a single infected cell) is $R_0 = b/(d + a)$. The establishment probability $P_{LR}$ is the solution to $R_0(1 - e^{-\lambda_0}) - P_{LR} = 0$. The total LR decay rate in the absence of viral replication is $\lambda_0$. If there are $Z$ cells in the LR, then the number of cells reactivating per day is $A = 2aZ$.

Analysis of the model to determine the four key parameters ($b$, $a$, $r$, $P_{LR}$), and rapidly compute survival curves is provided in SI Materials and Methods. A script for computation of survival curves is also provided at www.danielrosenbloom.com/reboundtimes.

Parameter Estimation. The half-life of latently infected cells has been estimated to be approximately $\tau_{LR} = 44$ mo (3, 4). The resulting value of $\lambda_0 = \ln(2)/\tau_{LR}$ is centered at $5.2 \times 10^4$ d$^{-1}$, and we construct a distribution of values based on ref. 3. This value represents the net rate of LR decay during suppressive therapy, considering activation, death, homeostatic proliferation, and (presumably rare) events where activated CD4$^+$ T cells reenter a memory state. The net infection growth rate $r$ describes the rate of exponential increase in viral load once infection has been reseeded. The LR reactivation rate $A$ is the number of cells exiting the LR per day, before LRA therapy. $A$ and $r$ were jointly estimated from the dynamics of viral load during treatment interruption trials in which there was no additional reservoir-reducing intervention (5, 58); in particular, infection growth immediately following rebound is sensitive to $r$, whereas the time to rebound is sensitive to $A$. Absent reservoir reduction, observed rebound dynamics are insensitive to $P_{LR}$, and so this parameter was estimated independently from population genetic models (59, 60) relating observed rates of selective sweeps and emergence of drug resistance to variance in the viral offspring distribution (SI Materials and Methods).

Simulation of the Model. We use the Gillespie algorithm to track the number of latently and actively infected cells in a continuous time stochastic process. The initial number of latent cells is $Z(0) = \text{Binomial}(N_{LR(0)}, q)$, where $N_{LR(0)}$ is pretreatment LR size and $q$ is efficacy of LRA treatment (fraction of cells remaining). The initial number of actively infected cells $Y(0)$ is then chosen from a Poisson distribution with parameter $a \lambda_0/d_a$ (corresponding to the immigration–death equilibrium of the branching process). The simulation proceeds until the number of actively infected cells reaches the threshold for clinical detection given by a viral load of 200 copies per mL (equivalent to $Y = 3 \times 10^5$ cells total) or until no active or latent cells remain. Because stochastic effects are important only for small $Y$, we switch to faster deterministic numerical integration when $Y$ reaches a level where extinction probability is very low ($<10^{-6}$). For each $q$ value we perform $10^4$ to $10^5$ simulations.

Simulations are seeded with values of the key parameters ($b$, $a$, $r$, $P_{LR}$), which may be either the point estimates or random numbers sampled from the distributions in Table 1. We then back out values of model-specific parameters consistent with the sampled key parameters. In general, we use a pretherapy LR size of $N_{LR(0)} = 10^6$ cells to get $a = ALR_{LR}$. We then have $d_a = \delta - a$. As detailed in SI Materials and Methods, sampling $P_{LR}$ requires first

---

**Materials and Methods**

**Basic Stochastic Model.** The basic model of reservoir dynamics and rebound tracks two cell types: productively infected activated CD4$^+$ T cells and latently infected resting CD4$^+$ T cells. The model can be described formally as a two-type branching process, in which four types of events can occur (Fig. 1):
and

Proc Natl Acad Sci USA
95(15):

Proc Natl Acad Sci USA
Curr HIV Res

We thank Y.-C. Ho, S. A. Rabi, L. Shan, and G. Laird for

AIDS

www.pnas.org/cgi/doi/10.1073/pnas.1406663111

cell infection.

d
487(7408):482

Trends Immunol

(St. Louis), Abstract 75LB.

Nat Med

24(18):2803

3(8):e122.

b
cure.

t cell reservoir for HIV in patients on HAART.

T cell reservoir for HIV in patients on HAART.


52. Fletcher CV, et al. (2014) Persistent HIV-1 replication is associated with lower antiretroviral

56. Xing S, et al. (2011) Disulfiram reactivates latent HIV-1 in a Bcl-2-transduced primary

37. Yukl SA, et al. (2013) Challenges in detecting HIV persistence during potentially cu-


40. Stöhr W, et al. (2013) Duration of HIV-1 viral suppression on cessation of antiretroviral


45. Gandhi RT, et al. (2011) Raltegravir intensification for persistent low-level HIV-1 viremia in


49. Clatworthy MC, et al. (2011) Quantitative polymerase chain reaction assay for measurement

50. Das S, et al. (2014) A single-copy assay for the quantification of HIV-1 DNA in latently


52. Fletcher CV, et al. (2014) Persistent HIV-1 replication is associated with lower antiretroviral

53. Lear SA, et al. (2013) Challenges in detecting HIV persistence during potentially cu-


56. Xing S, et al. (2011) Disulfiram reactivates latent HIV-1 in a Bcl-2-transduced primary


