Human Immunodeficiency Virus Rebound after Suppression to <400 Copies/mL during Initial Highly Active Antiretroviral Therapy Regimens, according to Prior Nucleoside Experience and Duration of Suppression

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This study evaluated 1433 human immunodeficiency virus (HIV)–infected patients starting highly active antiretroviral therapy (HAART), 409 (28%) of whom had prior nucleoside experience and achieved an HIV load of <400 copies/mL by 24 weeks of therapy. Three hundred seven patients experienced virus rebound during a total of 2773.3 person-years of follow-up. There was a higher rate of virus rebound among the patients with pre-HAART nucleoside experience (relative hazard [RH], 2.86; 95% confidence interval, 2.22–3.84; $P < .0001$) and a decreasing rate of virus rebound with increasing duration of virus suppression (i.e., time since achieving a virus load of <400 HIV RNA copies/mL) among both the nucleoside-experienced and naive patients ($P < .0001$), but the difference between the groups persisted into the third year of follow-up ($P = .0007$). Even patients who had experienced <2 months of nucleoside therapy before beginning HAART had an increased risk of virus rebound (RH, 1.95; $P = .009$). It appears that only a small period of pre-HAART nucleoside therapy is sufficient to confer a disadvantage, in terms of risk of virus rebound, that persists for several years.

Human immunodeficiency virus (HIV)–infected patients who experience monotherapy or dual therapy with nucleosides before receiving highly active antiretroviral therapy (HAART) tend to experience a poorer virus load response, compared with patients who are drug naive at the time of starting HAART [1–7]. Even for patients in whom virus suppression to below the limit of assay quantification is initially achieved, the subsequent rate of virus rebound is higher among nucleoside-experienced patients. To investigate the phenomenon in more detail, we combined data for patients starting HAART regimens in 2 large clinic cohorts. We aimed (1) to estimate over what periods of prolonged virus suppression during HAART the difference in rebound rate between naive and nucleoside-experienced patients persists and (2) to assess how the rate of rebound relates to the length of prior nucleoside experience, whether the nucleosides are changed at start of HAART, and whether there was prior use of monotherapy or dual therapy or both.

Methods

The Goethe Universität Clinic (Frankfurt, Germany) and the Royal Free Clinic (London, United Kingdom) cohorts collect data as a part of routine care of patients with HIV who attend these clinics [5, 8]. The available data include demographics, HIV exposure information, detailed treatment history, CD4 cell counts, and plasma virus load, in addition to occurrences of all AIDS-defining diseases. For this analysis, we selected all patients who started their first HAART regimen and achieved virus suppression, defined as a virus load of <400 HIV RNA copies/mL (measured using a Roche polymerase chain reaction–based method [Roche Molecular Systems]), by 24 weeks. HAART was defined as a ≥3-drug regimen, including 2 non-abacavir nucleosides plus at least either a protease inhibitor, a nonnucleoside reverse-transcriptase inhibitor, or abacavir.

Virus rebound was defined as 2 consecutive virus load measurements of >400 HIV RNA copies/mL, with the date of rebound being the date of the first of these measurements. If a patient was known to have interrupted or stopped all antiretroviral therapy at

Table 1. Demographic characteristics of the 1433 patients included in the Royal Free Centre for HIV Medicine and Goethe Universität Clinic cohorts.

Characteristic	All patients	Naive patients ($n = 1024$)	Nucleoside experienced patients ($n = 409$)
Location			
Frankfurt	954 (66)	703 (69)	251 (61)
London	479 (33)	321 (31)	158 (39)
Female	327 (23)	232 (23)	95 (23)
Route of HIV exposure			
Homosexual sex/men	763 (53)	535 (52)	228 (56)
IDU	166 (12)	120 (12)	46 (11)
Heterosexual sex	413 (29)	300 (29)	113 (28)
Other	91 (6)	69 (7)	22 (5)
Age, median years (25th–75th percentiles)	36 (31–43)	36 (31–42)	36 (31–44)
CD4 cell count at beginning of HAART, median cells/mm^3 (25th–75th percentiles)	189 (80–309)	200 (79–317)	162 (80–293)
Virus load at beginning of HAART, median 10^3 copies/mL (25th–75th percentiles)	135 (35–470)	195 (55–575)	43 (8–185)
Specific non-abacavir nucleosides			
Zidovudine/lamivudine	813 (57)	674 (67)	139 (34)
Stavudine/lamivudine	377 (26)	225 (22)	152 (37)
Stavudine/didanosine	141 (10)	54 (5)	87 (21)
Other	102 (7)	71 (6)	31 (8)
Other drugs			
Abacavir	179 (13)	166 (16)	13 (3)
Nevirapine	294 (21)	186 (18)	108 (26)
Efavirenz	167 (12)	156 (15)	11 (3)
Indinavir	475 (33)	303 (30)	172 (42)
Ritonavir	227 (16)	166 (16)	61 (15)
Nelfinavir	243 (17)	196 (19)	47 (12)
Saquinavir	54 (4)	36 (4)	18 (4)

NOTE.   Data are no. (%) of patients, except where noted. HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; IDU, injection drug use.

the time of the virus load rebound, his or her follow-up was right-censored at this point, and, thus, the patient was not considered to have a virus rebound end point. From 1998, virus load was measured with an assay with a lower quantification limit of 50 HIV RNA copies/mL, instead of 400 HIV RNA copies/mL, so we also analyzed a subgroup of patients who were known to have achieved a virus load of <50 HIV RNA copies/mL.

Kaplan-Meier plots and log-rank tests were used to describe and compare the proportions of patients with virus rebound over time. Cox models were used to consider the independent effect of various factors on the risk of virus rebound. We also calculated the incidence rates of virus rebound (number of patients with rebound/number of person-years at risk) in different periods of follow-up and according to prior nucleoside experience. Differences between groups were assessed using Poisson regression [9]. Statistical analysis was done using SAS software (version 6.12; SAS Institute).

Results

In total, 1433 patients were included in the analysis. Details of the demographic breakdown, virus load, and CD4 cell count at the start of HAART and of the drugs in the HAART regimen are given in table 1. Although, in most cases, the non-abacavir nucleoside drugs in the HAART regimen were lamivudine and either zidovudine or stavudine, the other drugs in the regimen were diverse, with indinavir, used by 475 patients (33%), being

the most common; 1024 patients (72%) were drug naive at the start of HAART. Details of previous therapy for the 409 patients (28%), who had experienced nucleosides, are given in table 2. Among these 409 patients, antiretroviral therapy was started a median of 15 months before HAART, but with only a median of 10 of these months actually spent receiving therapy. One hundred twenty-eight patients (31%) had stopped the nucleoside therapy by the time HAART was initiated; 31 (8%) had stopped for >1 year. Most patients had experienced dual nucleoside therapy, about half of whom also had experienced a period of monotherapy; 60% (245) of patients had experienced only 1 or 2 nucleosides, but 17% (70) had experienced ≥4. Nearly half ($n = 193$ [47%]) did not start a new, non-abacavir nucleoside when initiating HAART; only 94 (23%) started 2 new, non-abacavir nucleosides. We also looked at the median date of starting HAART and the percentage who had interrupted nucleoside therapy prior to starting HAART. For patients with ≥5 years of nucleoside therapy, the median date of starting HAART was November 1996, with 33% interrupting therapy; for those with 3–5 years of nucleoside therapy, the median date of starting HAART was March 1997, with 30% interrupting therapy; for those with 2–3 years of nucleoside therapy, the median date of starting HAART was April 1997, with 29% interrupting therapy; for those with 1–2 years of

nucleoside therapy, the median date of starting HAART was June 1997, with 30% interrupting therapy; for those with 6–12 months of nucleoside therapy, the median date of starting HAART was January 1997, with 31% interrupting therapy; for those with 2–6 months of nucleoside therapy, the median date of starting HAART was December 1996, with 36% interrupting therapy; and for those with >2 months of nucleoside therapy, the median date of starting HAART was August 1997, with 24% interrupting therapy.

Overall, there was a median of 1.65 years of follow-up among the 1433 patients before virus rebound occurred or, if rebound did not occur, before the last virus load was measured; there were 2773.3 person-years in total. Virus load was measured with a median frequency of 4.3 times/year in the Royal Free Clinic cohort and 7.5 times/year in the Goethe Universität Clinic Cohort. Figure 1 shows the Kaplan-Meier estimates of the percentage of patients with virus rebound, by years from initial virus load suppression to <400 HIV RNA copies/mL, according to prior nucleoside experience; 307 patients experienced virus rebound. There was a markedly higher percentage of patients who experienced virus rebound among the patients with prior nucleoside experience, compared with those who were drug naive at the start of HAART ($P < .0001$, log rank test). It also appears from these results that there is a decreasing tendency for virus rebound with increasing duration of virus suppression. This can be seen more clearly in table 3, which shows rates of virus rebound according to the time since virus load declined to <400 HIV RNA copies/mL and prior nucleoside experience. Among both naive and nucleoside-experienced patients, there is a highly significant trend toward a lower rate of virus rebound with increasing duration of virus suppression ($P < .0001$). Table 3 also shows rate ratios for each category of time with virus suppression. There is a statistically significantly higher rate of rebound among nucleoside-experienced patients even after 2–3 years of virus suppression. There remains a lower rate in naive patients after year 3, but this result was not statistically significant, so it is not possible to say whether the difference in rebound rate persists for this length of time.

We fitted a Cox model to assess factors associated with virus rebound. For this model, time zero was the date of virus suppression to <400 HIV RNA copies/mL (as in the Kaplan-Meier plot in figure 1). The effect of the duration of virus suppression was not assessed directly, but this effect is incorporated within the underlying hazard function of the Cox model. Other variables included were specific drugs in the regimen, age, risk group, calendar year, and sex. Nucleoside experience status (naive vs. experienced) was significantly associated with the rate of rebound after adjustment for these other covariates (relative hazard, [RH], 2.86 for nucleoside-experienced patients, compared with naive patients; 95% confidence interval [CI], 2.22–3.84; $P < .0001$). Additional adjustment for the virus load at start of HAART (RH, 1.1 per 1 log higher; $P = .2$), CD4 cell

Table 2. Characteristics of the 409 nucleoside-experienced patients in the Royal Free Centre for HIV Medicine and Frankfurt Clinic cohorts.

Characteristic	Value
Time since start of antiretroviral therapy, median months (25th–75th percentiles)	15 (7–36)
Duration of nucleoside use, median months (25th–75th percentiles)	10 (4–26)
Not receiving therapy	128 (31)
Not receiving therapy for >12 months	31 (8)
Therapy experienced	
Monotherapy only	29 (7)
Dual therapy only	172 (42)
Both monotherapy and dual therapy	208 (51)
No. of nucleosides ever experienced	
1	29 (7)
2	216 (53)
3	94 (23)
≥4	70 (17)
No. of new nucleosides started, excluding abacavir	
0	193 (47)
1	122 (30)
2	94 (23)
Specific drugs	
Zidovudine	373 (91)
Didanosine	74 (18)
Zalcitabine	161 (39)
Stavudine	126 (31)
Lamivudine	297 (73)

NOTE. Data are no. (%) of patients, except where noted.

count at the start of HAART (RH, 0.83 per 100 cells/mm³ higher; $P < .0001$), and CD4 cell count at the time virus suppression was achieved (RH, 0.88 per 100 cells/mm³ higher; $P < .0001$) made almost no difference to this estimate; the latter 2 variables were not included in the same model because they are highly correlated. In a further model, we categorized the nucleoside-experienced patients according to the length of previous nucleoside experience. RHs after adjustment for other factors in the model are shown in figure 2. There was a 1.96-fold (95% CI, 1.19–3.23; $P = .009$) increased risk of rebound, even for the group of patients ($n = 62$) who had experienced <2 months of nucleoside therapy before beginning HAART. There was no apparent trend for increasing RH after ~6 months of pre-HAART nucleoside experience.

We also fitted a Cox model in which time zero was 1 January 1995, so that the time with virus suppression could be assessed. Follow-up times were left-truncated until the date the patient achieved virus suppression. This confirmed that the association between the duration of virus suppression and the rate of rebound was independent of the other covariates mentioned above ($P < .0001$).

Further Cox models were fitted for the nucleoside-experienced patients only. Covariates considered included the number of new nucleosides (i.e., drugs that the patient had never taken before) started at the time of HAART, whether the nucleosides had been stopped at the time of starting HAART, the number of prior nucleosides experienced, and whether monotherapy only, dual therapy only, or both had been used. None of these
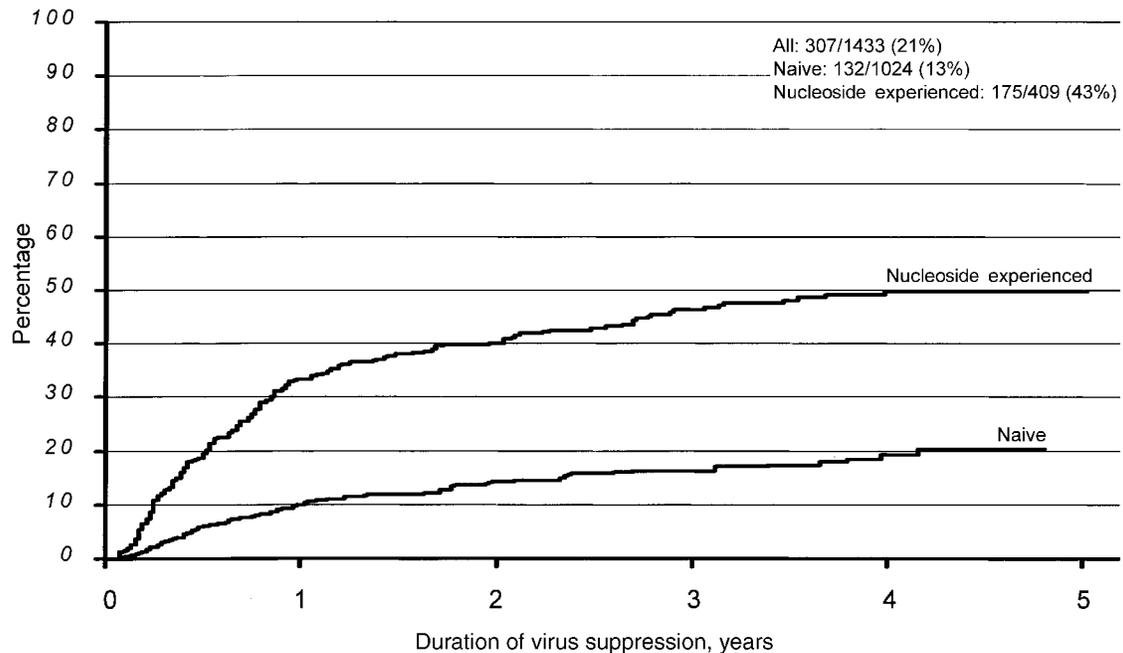
Figure 1. Kaplan-Meier estimates of the percentage of subjects with human immunodeficiency virus (HIV) rebound, by the duration of virus suppression (i.e., time since achieving a virus load of <400 HIV RNA copies/mL) during highly active antiretroviral therapy (HAART), according to pre-HAART nucleoside experience.

covariates was significantly associated with the rate of virus rebound. In a model that included only drug-naive patients and those nucleoside-experienced patients for whom 2 new nucleosides were started at the start of HAART, there was a significantly higher rate of rebound in the latter group (RH, 3.23; 95% CI, 1.96–5.00; $P < .0001$).

We also assessed the RH of virus rebound to >400 HIV RNA copies/mL associated with nucleoside experience status in the subgroup of 848 patients (105 with rebound) for whom a virus load of <50 HIV RNA copies/mL was measured. A similar value was found as in the main analysis (RH, 3.33; 95% CI, 2.13–5.26; $P < .0001$).

Discussion

It is well established that patients who had previously taken nucleoside monotherapy or dual therapy before starting

HAART tend to experience a poorer virologic response to HAART [1–7], which is thought to relate to build-up during nucleoside therapy of virus subspecies that are partially resistant to drugs in the HAART regimen [10, 11]. This phenomenon has been illustrated in the Merck 035 trial [12], in which patients randomly assigned to receive indinavir alone or zidovudine plus lamivudine before starting HAART with the 3 drugs together experienced a poorer long term virologic response than did patients who initiated all 3 drugs simultaneously.

Our results extend earlier findings in several ways. First, we have confirmed that, even among patients who have achieved virus suppression, there is a greater tendency for virus rebound to occur among patients with pre-HAART nucleoside experience, compared with patients who are drug naive. This may relate to the presence of archived virus subspecies that are partially or wholly resistant to the HAART regimen. Such archives exist, for example, in long-lived latently infected cells [13]. Per-

Table 3. Human immunodeficiency virus (HIV) rebound, by the duration of virus suppression (i.e., virus load <400 HIV RNA copies/mL) and prior nucleoside experience.

Characteristic	Data, by duration of virus suppression, years				P for trend over time
	<1	1–2	2–3	≥3	
Patient group					
Naive	91/850.4 (0.107)	25/548.5 (0.046)	9/337.2 (0.027)	7/214.0 (0.033)	<.0001
Nucleoside experienced	128/314.0 (0.408)	23/205.0 (0.112)	17/156.9 (0.108)	7/147.3 (0.048)	<.0001
Comparison, rate ratio (P)	3.81 (<.0001)	2.44 (.002)	4 (.0007)	1.46 (.48)	

NOTE. Data are no. of patients experiencing virus rebound/no. of person-years of experience (yearly rate of virus rebound), except where noted.
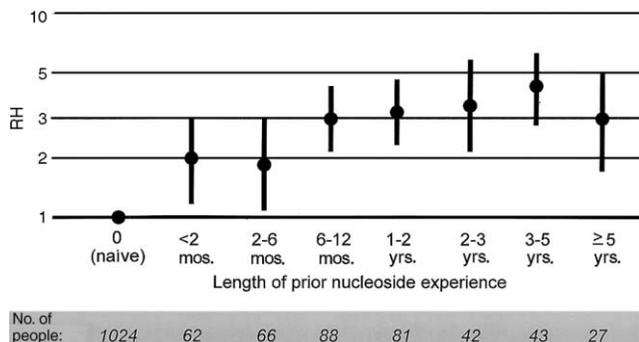
Figure 2.　Relative hazards (RHs) of human immunodeficiency virus (HIV) rebound after suppression to <400 HIV RNA copies/mL during highly active antiretroviral therapy (HAART), according to the duration of nucleoside experience before HAART, adjusted for specific drugs in the regimen, age, risk group, calendar year, and sex. Bars represent 95% confidence intervals.

haps only when such cells become activated at some point in time will the resistant virus they harbor be released. Although relatively few nucleoside experienced patients in our analysis started 2 new nucleoside drugs at the time of starting HAART ($n = 94$), we found that, even in this group, there was a higher rate of virus rebound than among patients who were nucleoside naive when starting HAART. This perhaps suggests that there is more cross-resistance between nucleoside analogue drugs than has been appreciated.

The difference in rate of virus rebound we observed between pre-HAART nucleoside-experienced and naive patients likely is related to the nucleoside experience itself and not to some other confounding factor that is different between the 2 groups. This is because the effect is very large and highly significant; the difference in initial virus load response to therapy has been observed in many studies [1–7], and a likely underlying mechanism (resistance) has been identified. Furthermore, all patients in both groups appear to have been at least initially adherent to therapy, because an initial virus load response was achieved, so major confounding due to differences in adherence seems to be improbable. We also found that the difference persisted (and was not diminished) after adjustment for calendar period, specific drugs used, age, HIV risk group, and sex.

The second key finding is that there is some increased risk of virus rebound present, even among patients with <2 months of prior nucleoside experience before starting HAART. This finding may indicate that there is a rapid time scale over which archives of resistant virus could accumulate, but, whatever the underlying reason, the finding may have consequences for the use of short-term monotherapy regimens in pregnant women [14]. This finding also suggests that clinical trials of drug-naive individuals that allow participants to have 1–6 months of prior experience with zidovudine monotherapy or other nucleosides may result in underestimation of the effect of regimens in truly naive individuals. Although we have only been able to ascertain that <2 months

of pre-HAART nucleoside therapy is sufficient to lead to a disadvantage in terms of long-term risk of virus rebound, with more data on a larger number of individuals with short periods of pre-HAART nucleoside therapy, it may be possible in future analyses to further characterize how many weeks of such therapy are sufficient to produce this disadvantage. It is unclear to what degree interpretation of our findings can be extended to situations where short periods of monotherapy of drugs other than nucleosides are given before HAART, but this does happen as part of development of these new drugs, in order to isolate the effect of such drugs.

A third important finding is that, among both naive and nucleoside-experienced patients, there is a decreasing rate of virus rebound with increasing duration of virus suppression. This had previously been shown for naive patients starting HAART in the Goethe Universität cohort [15]. There could be several explanations for this. There could be some kind of selection effect, whereby patients who are most adherent to therapy, who experience the least degree of drug toxicity, who achieve the most consistently high drug levels, who have the fewest preexisting mutations associated with drug resistance, or who have some other biological advantage are gradually selected out. Another possible explanation is that the declining rate reflects a declining rate of appearance of new productively infected cells, perhaps as the pool of latently infected cells becomes reduced in size. However, the rate of decline in the pool of latently infected cells has been found to be very small [13].

Finally, despite the decreasing tendency for virus rebound over time, the disadvantage experienced by patients who had taken prior nucleoside therapy appears to last for at least up to 3 years with virus suppression, perhaps longer. This would be consistent with the concept that the archived resistant virus in nucleoside-experienced patients is in cells that may not become activated and release virus for at least 3 years. This is certainly consistent with estimates of the life-span of such cells [13]. Indeed, those estimates would lead to the prediction that the disadvantage for nucleoside-experienced patients will last considerably longer than 3 years.

In summary, the rate of rebound declines substantially over increasing time with virus suppression during HAART in both nucleoside-experienced and naive patients. However, the markedly increased rate of virus rebound experienced by patients who took nucleosides before receiving HAART persists even after up to 3 years of prolonged virus suppression. This disadvantage seems to be apparent even among patients with <2 months of prior nucleoside use before beginning HAART.

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