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Evaluation of Rapid Progressors in HIV Infection as an Extreme Phenotype

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Design: Rapid CD4 cell loss represents an HIV phenotype used to identify causal variants of accelerated disease progression. The optimal rate and threshold for identifying this extreme phenotype in recently infected individuals is unclear.

Methods: Using a cohort of patients with known dates of HIV-1 seroconversion (SC), CASCADE (Concerted Action on SeroConversion on AIDS and Death in Europe), we identified proportions experiencing nadir CD4 cell levels within 1 year of SC, and assessed their mean AIDS-free survival time at 10-year follow-up and hazard of AIDS/death, compared with those whose CD4 remained >500 cells per cubic millimeter. Follow-up was censored at December 31, 1996 to avoid bias due to combination antiretroviral therapy initiation.

Results: Of 4876 individuals, 2.8%, 7.3%, and 24.9% experienced ≥1 CD4 <100, 200, and 350 cells per cubic millimeter, respectively, within 1 year of SC. Minimum CD4 levels of 30, 166, 231, and 506 cells per cubic millimeter were experienced during this period by 1%, 5%, 10%, and 50% of individuals, respectively. Mean (95% confidence interval) AIDS-free survival at 10 years follow-up was 2.9 (2.3 to 3.6), 5.5 (5.0 to 6.1), 6.7 (6.5 to 7.0), 7.4 (7.2 to 7.6), and 8.1 (7.9 to 8.3), for those with minimum counts ≤100, 100–200, 200–350, 350–500, >500 cells per cubic millimeter, respectively. Using counts of >500 cells per cubic millimeter as reference, the hazard ratios (95% confidence interval) of AIDS/death were 15.0 (11.9 to 18.9), 3.6 (2.9 to 4.5), 2.1 (1.8 to 2.4), and 1.5 (1.3 to 1.7), respectively. The hazard ratio increased to 37.5 (26.5 to 53.1) when a minimum CD4 count <100 was confirmed within 1 year of SC.

Conclusion: At least 1 CD4 ≤100 cells per cubic millimeter within the first year of SC identifies a rare group of individuals at high risk of disease progression and could form the basis for defining the rapid progressor phenotype.

Key Words: HIV, rare phenotype, disease progression, genetics

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INTRODUCTION

Rapid HIV disease progression is an extreme HIV phenotype, although there is little consensus on a definition. Differences in HIV disease progression can be assessed by variability in biomarkers related to HIV disease, the 2 most common of which being CD4 and HIV-RNA. Variability in HIV-RNA, specifically low-circulating HIV-RNA, has defined those with slow disease progression, termed long-term nonprogressor or elite controller phenotypes, which are of particular importance to vaccine studies.[1–10] Rapid progression, however, is equally important as it also contributes to our understanding of early risk factors of disease progression. This may in turn help optimize the frequency of clinical monitoring and antiretroviral therapy initiation.

In addition to the well-documented relationship between slow disease progression and low HIV-RNA, there is also known variation in CD4 levels at or shortly after seroconversion (SC). A number of studies to date have defined rapid progression based on various levels of immunosuppression,[11–21] but it is not yet clear if this variability in CD4, 1 early measure or consecutive low CD4 measurements do, indeed, constitute rapid progression and how this rare phenotype should be defined.

The CASCADE (Concerted Action on SeroConversion on AIDS and Death in Europe) Collaboration, of HIV-positive individuals followed-up since HIV SC offers a unique opportunity to evaluate HIV rapid progression. Using data from CASCADE, we aim to document low CD4 near SC and examine HIV rapid disease progression. This work provides

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For a complete list of CASCADE Collaboration in EuroCoord, see Appendix 1.

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the basis for choosing a definition appropriate to the objectives of future research on this extreme phenotype.

METHODS

Study Population

We used data from the CASCADE 2011 data release in EuroCoord (www.EuroCoord.net), which consists of 25,629 seroconverters from 28 cohorts across Europe, Canada, Australia, and sub-Saharan Africa.[22] Date of HIV SC were estimated by various methods, most commonly as the midpoint between the last documented HIV-negative and the first positive HIV antibody test dates with an interval of <3 years between the 2 test dates (85%). For the remainder, date of SC was estimated through laboratory evidence of SC (polymerase chain reaction positivity in the absence of HIV antibodies or antigen positivity with fewer than 4 bands on Western blot) (13%), or as the date of a SC illness (2%) with both an earlier documented negative and a later positive HIV test not more than 3 years apart. All cohorts contributing data to CASCADE received approval from their individual ethics review boards.

Rapid Progression

Because of the inconsistency of definitions described in the literature,[11–21] with nadir CD4 cell counts ranging between 200 and 500 cells per cubic millimeter and follow-up ranging between 6 months and 8 years, we sought to identify those at highest risk of disease progression by severity of immunosuppression in early infection. We evaluated the frequency of low CD4 counts during the first year after SC and estimated the mean survival time at 10 years of follow-up and hazard of AIDS/death by nadir CD4 levels compared with individuals whose CD4 measurements remained above such levels during that period.

Statistical Methods

For all estimates, we considered only data from individuals who met the requirements for length of follow-up and minimum number of CD4 measurements. More specifically, only individuals with at least 1 CD4 count measured within the first year of SC could contribute information to assessing the risk associated with experiencing any specific nadir CD4 cell count within 1 year.

To provide estimates of the prevalence of low CD4 near SC, we calculated proportions experiencing various nadir CD4 levels within the first year of SC by mode of HIV transmission and age categories and plotted the cumulative proportion of individuals experiencing different nadir CD4 levels.

We used Cox proportional hazards models to estimate the relative hazard of AIDS/death among eligible individuals. As the nonproportionality assumption was not met in 3 of the 4 models, we used log-rank P values.[23] We estimated restricted mean AIDS-free survival times (the area under the length of AIDS-free survival curves) at 10 years of follow-up using clinically relevant CD4 categories (<100, 100–200, 200–350, 350–500, and >500 cells/mm^3) using pseudovalues, as described previously.[23,24] The follow-up time of 10 years was chosen as this is the median time to AIDS in the pre–combination antiretroviral therapy (cART) era for individuals infected between 25 and 35 years of age and was close to the last observed event time.[25] We also used fractional polynomials to explore the relationship of nadir CD4 measurements to the hazard of AIDS/death and restricted mean AIDS-free survival.[26] For all models, we adjusted for the following potential confounders: sex, mode of HIV transmission, age at SC, and year at SC. Age and year of SC were modeled using restricted cubic splines with 3 knots.[27] For all analyses, follow-up was censored at the earliest of AIDS or death date or on December 31, 1996 to avoid bias because of treatment initiation. AIDS was defined using the European case definition, which excludes CD4 <200 cells per cubic millimeter.[28]

In sensitivity analyses, we investigated the proportions experiencing nadir CD4 measurements within 6 months of SC

TABLE 1. Baseline Characteristics for (A) 4876 Individuals With ≥1 CD4 Cell Measurement(s) Within 1 year of SC Included in Analysis and (B) 6084 Individuals Not Included in the Analysis but Seroconverting in the Pre-cART Era Using the CASCADE Data Set

	A	B
Risk category, n (%)		
MSM	2564 (53)	2612 (43)
MSW	1026 (21)	1741 (29)
IDU	1085 (22)	1243 (21)
Other/unknown	201 (4)	486 (7)
Sex, n (%)		
Male	3798 (78)	4638 (76)
Female	1078 (22)	1446 (24)
SC year		
Median (IQR), yrs	1992 (1989–1994)	1991 (1988–1994)
SC age		
Median (IQR), yrs	29 (25–35)	28 (24–33)
<20, n (%)	239 (5)	454 (7)
≥20–30, n (%)	2481 (51)	3377 (56)
≥30–40, n (%)	1479 (30)	1613 (27)
≥40, n (%)	677 (14)	640 (11)
Geographical origin, n (%)		
Europe	3652 (78)	4765 (75)
Africa	117 (3)	170 (2)
Americas	50 (1)	77 (1)
Unknown/Other	1057 (18)	1072 (22)
Time from SC to nadir CD4: median (IQR), mo	7.4 (4.5–9.8)	—
Time from SC to first CD4: median (IQR), mo	5.2 (3.0–7.9)	—
Number of CD4 counts: median (IQR)	2 (1–2)	—
Follow-up time: median (IQR)	3.8 (1.9–6.1)	—

IDU, injection drug users; MSM, men who have sex with men; MSW, heterosexual contact.

and the hazard of AIDS/death observed by these minimum levels. We also investigated if confirmed CD4 measurements (ie, 2 counts) had an impact on the proportion, mean AIDS-free survival times, and hazard of AIDS/death within 6 months and 1 year of SC. Additionally, as the individuals in CASCADE are geographically diverse, we stratified all analysis by geographical origin. Analyses were conducted using Stata/IC 13.0.

RESULTS

Baseline Characteristics

Of 25,629 seroconverters, 20,753 were excluded for following reasons: 14,669 seroconverted after 1997, 6074 had no CD4 measurements within the first year of SC, 6 had an unknown AIDS date and 4 were <15 years of age at SC. Of the remaining 4876 individuals who were studied, 53% were men infected through sex between men (men who have sex with men), 21% through heterosexual contact, 22% through injection drug use, and the remainder were hemophiliacs or with unknown risk categories. The majority (78%) were male seroconverting at a median [interquartile range [IQR]) 29 (25–35) years old between 1982 and 1996. Median (IQR) time from SC to the lowest CD4 was 7.4 (4.5–9.8) months. Geographical origin was predominately European (78%) with few individuals from Africa (3%) and the Americas (1%) (Table 1). HIV subtype was missing for >80% of individuals in this analysis, but of those with known subtype, the data comprised mainly subtype B (>90%).

Baseline characteristics of the 6084 individuals seroconverting in the pre-cART era excluded from this analysis and the sensitivity analysis were similar to the 4876 individuals included in this analysis (Table 1) (see **Table S1, Supplemental Digital Content**, http://links.lww.com/QAI/A542).

CD4 Near SC

Median (IQR) initial CD4 count during the first year of SC was 550 (384–726). A total of 138 (2.8%), 356 (7.3%), and 1213 (24.9%) experienced at least 1 CD4 below 100, 200, and 350 cells per cubic millimeter, respectively, in the first year of SC (Fig. 1, Table 2). About 1%, 5%, 10%, and 50% of individuals experienced at least 1 CD4 <30, 166, 231, and 506 cells per cubic millimeter, respectively, within the first year of SC (Table 2). Higher CD4 cell levels were experienced by younger individuals and those infected through injection drug use (Table 2).

In sensitivity analyses, data were available from 2641, 2825, and 894 individuals with a confirmed CD4 within 1 year of SC, at least 1 CD4 within 6 months, and a confirmed CD4 within 6 months, respectively. Nadir CD4 percentiles remained qualitatively similar to those obtained from the main analysis (see **Table S2, Supplemental Digital Content**, http://links.lww.com/QAI/A542).

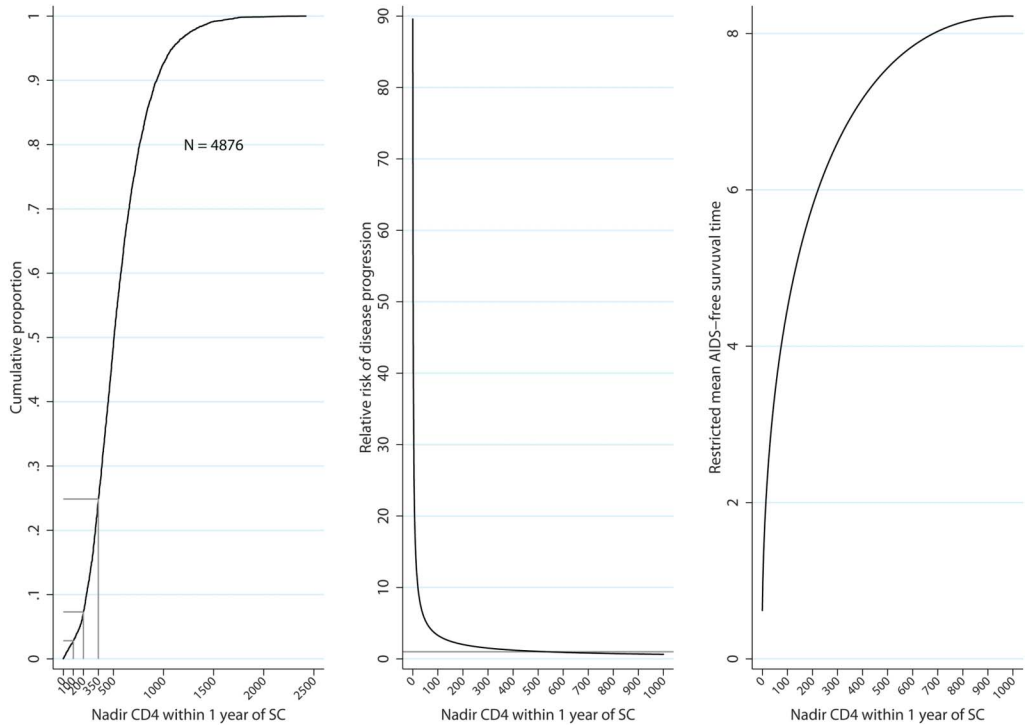

FIGURE 1. Cumulative proportions of nadir CD4 cell count (left hand panel), relative risk of AIDS/death compared with individuals whose CD4 counts remained at 500 cells per cubic millimeter (center panel), and mean AIDS-free survival time at 10 years follow-up (right hand panel) for individuals in CASCADE experiencing specific nadir levels within 1 year of SC during that period: all individuals seroconverted in the pre-cART era.

TABLE 2. Minimum CD4 Percentiles Within 1 year of SC by HIV Risk Group and Age Categories in the Pre-cART Era Using the CASCADE Data Set

	<20	≥20–30	≥30–40	≥40	Overall
1%					
MSM	165	49	27	30	35
IDU	244	53	22	185	40
MSW	154	19	27	23	27
OTH	—	65	0	24	19
Overall	156	49	24	24	30
5%					
MSM	220	168	156	124	161
IDU	318	210	141	240	200
MSW	257	168	135	95	156
OTH	—	136	48	64	112
Overall	257	180	148	118	166
10%					
MSM	270	237	223	213	224
IDU	338	269	220	339	265
MSW	300	255	220	190	225
OTH	—	240	182	192	200
Overall	307	250	220	204	231
25%					
MSM	342	352	340	315	340
IDU	439	425	343	364	405
MSW	440	370	350	268	350
OTH	—	404	289	328	318
Overall	389	378	340	311	352
50%					
MSM	450	500	487.5	441	484.5
IDU	597	600	510	419.5	583
MSW	560	519	491	430	501.5
OTH	—	546	500	474	502
Overall	530	535	491	440	506
75%					
MSM	638	676	650	614	654
IDU	778	825	766	725	815
MSW	840	732	722.5	603	751
OTH	667	726	800	612	721
Overall	717	736	680	612	705.5
90%					
MSM	—	875	842	830	856
IDU	947	1063	1045	1029	1057
MSW	997	979	970	824	950
OTH	842.5	977	1154	800	942
Overall	947	966	907	830	933
100%					
MSM	1250	1704	1875	1584	1875
IDU	1984	2105	1744	2420	2420
MSW	1453	2231	2156	1625	2231
OTH	—	1704	2068	1357	2068
Overall	1984	2231	2156	2420	2420

IDU, injection drug users; MSM, men who have sex with men; MSW, heterosexual contact; OTH, hemophiliacs or unknown.

Predicted Mean Survival Time at 10-Year Follow-Up

The AIDS-free survival expectancy at 10 years follow-up significantly increased as nadir CD4 count measured within the first year of SC increased (Fig. 1, Table 3). Compared with individuals experiencing nadir CD4 counts <100 cells per cubic millimeter within the first year of SC, those with nadir CD4 of 100–200, 200–350, 350–500, and >500 have an increased AIDS-free survival expectancy of 2.6 (1.7–3.4), 3.8 (3.1–4.45), 4.5 (3.8–5.2), and 5.2 (4.5–5.5) years, respectively, during the first 10 years of HIV infection.

In a sensitivity analysis, the AIDS-free survival expectancy was qualitatively similar to results in the main analysis, increasing as nadir CD4 count and confirmed minimum CD4 measurements increased within 1 year and the 6 months of SC (Table 3). Predicted mean survival at 10 years follow-up was qualitatively similar when stratifying by geographical origin (data not shown).

Risk of AIDS/Death by Nadir CD4

The risk of AIDS/death increased as nadir CD4 count measured in the first year decreased. For individuals experiencing at least 1 count <100 cells per cubic millimeter, there was a 15-fold increased risk of AIDS/death compared with those whose nadir CD4 count remained >500 cells per cubic millimeter. Hazard of AIDS/death was significantly higher for those with nadir counts <500 cells per cubic millimeter, (Fig. 1, Table 3).

In sensitivity analyses, the risk of AIDS/death was qualitatively similar to results in main analyses. As expected, however, the risk was greatly elevated for those experiencing confirmed counts, namely; hazard ratio (95% confidence interval) 15.0 (11.9 to 18.9) vs. 37.5 (26.5 to 53.1) for CD4 ≤100 cells per cubic millimeter and 3.6 (2.9 to 4.5) vs. 6.3 (4.5 to 8.8) for CD4 100–200 cells per cubic millimeter comparing a single minimum CD4 count with a confirmed CD4 count within 1 year of SC. This same pattern was observed when comparing nadir CD4 with a confirmed minimum CD4 count within 6 months of SC, (Table 3). There was a similar trend of higher risk of AIDS/death for lower CD4 cell counts when stratifying by geographical origin (data not shown).

DISCUSSION

Individuals experiencing 1 or more CD4 cell count ≤100 cells per cubic millimeter within the first year of SC provide a rare group (2.8%) of HIV-positive individuals at the highest risk of disease progression with remarkably short mean AIDS-free survival of 2.9 years. These results suggest that CD4 monitoring close to SC may play an important role in identifying those at highest risk of progression. In addition to this, individuals with at least 1 CD4 cell count ≤500 cells per cubic millimeter are at an increased risk of AIDS/death compared with individuals whose CD4 remain above 500 cells per cubic millimeter.

We have shown that low CD4 cell counts <100 cells per cubic millimeter near SC is rare, but low CD4 near SC can

TABLE 3. HR for Time to AIDS/Death by Nadir CD4 Measured Within 1 year of SC Using the CASCADE Data Set

	Within 6 Months of SC			Within 1 Year of SC		
CD4 Value, cells/mm^3	N (Fail)	HR (95% CI)*	Mean AIDS-Free Survival (95% CI)†	N (Fail)	HR (95% CI)*	Mean AIDS-Free Survival (95% CI)†
Nadir CD4						
≤100	58 (37)	12.8 (9.0 to 18.2)‡	3.1 (2.1 to 4.1)	138 (94)	15.0 (11.9 to 18.9)‡	2.9 (2.3 to 3.6)
100–200	97 (40)	2.9 (2.1 to 4.1)‡	5.6 (4.7 to 6.5)§	218 (91)	3.6 (2.9 to 4.5)‡	5.5 (5.0 to 6.1)§
200–350	409 (134)	2.3 (1.9 to 2.8)‡	6.3 (5.9 to 6.6)§	857 (267)	2.1 (1.8 to 2.4)‡	6.7 (6.5 to 7.0)§
350–500	607 (180)	1.6 (1.3 to 1.9)‡	7.0 (6.7 to 7.3)§	1185 (316)	1.5 (1.3 to 1.7)‡	7.4 (7.2 to 7.6)§
>500	1654 (359)	1‡	8.0 (7.8 to 8.1)§	2478 (501)	1‡	8.1 (7.9 to 8.3)§
Confirmed CD4						
≤100	14 (10)	49.3 (23.4 to 104.2)	2.1 (0.4 to 3.7)	64 (51)	37.5 (26.5 to 53.1)‡	1.8 (1.1 to 2.6)
100–200	33 (17)	7.4 (4.2 to 13.0)	3.6 (2.3 to 5.0)	92 (40)	6.3 (4.5 to 8.8)‡	4.9 (4.0 to 5.7)§
200–350	118 (36)	2.5 (1.7 to 3.7)	5.6 (4.9 to 6.4)§	342 (106)	2.4 (1.9 to 3.0)‡	6.3 (5.9 to 6.7)§
350–500	186 (61)	1.6 (1.2 to 2.3)	6.1 (5.5 to 6.7)§	577 (171)	1.8 (1.5 to 2.1)‡	6.8 (6.4 to 7.1)§
>500	543 (130)	1	7.3 (6.9 to 7.7)§	1566 (344)	1‡	7.9 (7.7 to 8.1)§

*Adjusted for sex, risk group, SC age, SC year, log-rank test $P < 0.001$.
†Mean AIDS-free survival at 10 years of follow-up.
‡Cox proportional hazards $P < 0.05$.
§Statistically greater than ≤100 cells per cubic millimeter category ($P < 0.001$).
CI, confidence interval; HR, hazard ratio.

have other research implications. Our definition can be useful for researching extreme phenotypes, particularly for genetic studies aiming to identify rare causal variants by looking at extreme ends of HIV disease progression.[29,30] In addition to genetic implications, low CD4 near SC can have impact on HIV incidence measurements. HIV incidence measures such as The Recent Incidence Testing Algorithm aim to identify individuals infected within 4–6 months of sampling but exclude individuals who have AIDS, on ART or are identified with low CD4 as these individuals have been shown to be misclassified as recently infected.[31] Our study suggests that up to 5% of the HIV-positive population tested in the first 6 months of SC will have a CD4 below 200 and thus would be misclassified as longstanding infection according to The Recent Incidence Testing Algorithm. These results suggest the need for an incidence estimate correction factor to account for low CD4 cell counts near SC.

Individuals experiencing confirmed low CD4 measurements <200 cells per cubic millimeter had more than a 2-fold increased risk of AIDS/death compared with minimum CD4 measurement alone. Although this may suggest that a confirmatory CD4 has a higher prognostic value of disease progression than a single CD4 alone, it is unusual for individuals to have a confirmed CD4 so close to SC, shown by our reduced numbers for this population. We were able to analyze repeated low CD4 measurements because these data were restricted to the pre-cART era; however, it is unlikely that in the cART era naive low confirmed CD4 measurements would be available, as all individuals with 1 CD4 <200 cells per cubic millimeter are recommended to be on treatment.

Subtype was missing for >80% of individuals in this analysis, and comprised mainly subtype B (90%), which compared with other HIV subtypes, has previously been shown to have different rates of CD4 cell levels near SC and CD4 rates of decline suggesting these results may not be generalizable to other HIV subtypes.[32] We stratified the analysis by geographical origin and the same trend of higher risk of disease progression with lower CD4 cell counts was observed, suggesting these results are generalizable in different global epidemics.

Our study has several strengths. First, the availability of SC estimation is essential to identifying individuals with rapid disease progression. Without laboratory evidence of SC, individuals entering care with low CD4 would be termed late presenters instead of rapid progressors.[33] Second, the availability of data in an era when ART was not used early in the course of disease allowed us to assess rapid progression without the interaction of ART on disease progression. In the cART era, individuals with CD4 <350 cells per cubic millimeter would be on cART and the impact of low CD4 <100 cells per cubic millimeter near SC would not be fully understood. Finally, the large sample size of our cohort allows us to compare between different possible combinations of this rare phenotype.

Our study has limitations. SC illness and HIV test intervals <31 days have been shown to be associated with faster disease progression,[34,35] suggesting our proportion and risk estimates could be overinflated because of the increased likelihood of individuals seeking care when experiencing SC illness, although the midpoint method of estimating SC was used for 85% of seroconverters. We were unable to test if rapid progressors are more likely to report SC illness, as this is unknown in >70% of the CASCADE data set. However, among 1481 individuals in our study with known SC illness status, > 50% of individuals reported of SC illness with CD4 count <350 cells per cubic millimeter, where <50% of individuals reported no SC illness in those with a CD4 count ≥350 cells per cubic millimeter (data not shown). Although our study only

investigates seroconverters, it has been shown that HIV progression, in particular CD4 decline, among seroconverters is similar to that of the general HIV-positive population suggesting our results are generalizable to the HIV-positive population.[36]

In conclusion, individuals with at least 1 CD4 ≤100 cells per cubic millimeter in the first year of SC are a rare and extreme group who are at a very high risk of rapid disease progression. Given that the HIV test intervals in this study are consistent with HIV testing guidelines,[37–40] our study allows clinicians to identify individuals at risk of progression at an early stage for whom immediate initiation of therapy may be indicated. This study has also helps to identify an extreme HIV phenotype that increases power to detect rare variants in causal viral and host genetics of rapid HIV disease progression. This may, in turn, lead to targeted treatments for individuals at the greatest risk of progression. We suggest future research use at least 1 CD4 ≤100 cells per cubic millimeter within 1 year of SC as a definition for rapid progression.

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APPENDIX 1

CASCADE Steering Committee: Julia Del Amo (Chair), Laurence Meyer (Vice Chair), Heiner C. Bucher, Geneviève Chêne, Osamah Hamouda, Deenan Pillay, Maria Prins, Magda Rosinska, Caroline Sabin, Giota Touloumi.

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