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ORIGINAL PAPER

Comparison of Self-report to Biomarkers of Recent HIV Infection: Findings from the START Trial

Katherine E. Schlusser[1] · Shweta Sharma[2] · Pola de la Torre[3] · Giuseppe Tambussi[4] · Rika Draenert[5] · Angie N. Pinto[6] · Julia A. Metcalf[7] · Danielle German[8] · James D. Neaton[2] · Oliver Laeyendecker[1,9] · for the INSIGHT START Study Group

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Abstract
Identifying individuals with recent HIV infection is critical to research related to viral reservoirs, outbreak investigations and intervention applications. A multi-assay algorithm (MAA) for recency of infection was used in conjunction with self-reported date of infection and documented date of diagnosis to estimate the number of participants recently infected in the Strategic Timing of AntiRetroviral Treatment (START) trial. We tested samples for three groups of participants from START using a MAA: (1) 167 individuals who reported being infected ≤ 6 months before randomization; (2) 771 individuals who did not know their date of infection but were diagnosed within 6 months before randomization; and (3) as controls for the MAA, 199 individuals diagnosed with HIV ≥ 2 years before randomization. Participants with low titer and avidity and a baseline viral load > 400 copies/mL were classified as recently infected. A significantly higher percentage of participants who self-reported being infected ≤ 6 months were classified as recently infected compared to participants diagnosed ≥ 2 years (65% [109/167] vs. 2.5% [5/199], $p < 0.001$). Among the 771 individuals who did not know their duration of infection at randomization, 206 (26.7%) were classified as recently infected. Among those diagnosed with HIV in the 6 months prior to enrollment, the 373 participants who reported recent infection (n = 167) or who had confirmed recent infection by the MAA (n = 206) differed significantly on a number of baseline characteristics from those who had an unknown date of infection and were not confirmed by the MAA (n = 565). Participants recently infected by self-report and/or MAA were younger, more likely to be Asian, less likely to be black, less likely to be heterosexual, more likely to be enrolled at sites in the U.S., Europe or Australia, and have higher HIV RNA levels. There was good agreement between self-report of recency of infection and the MAA. We estimate that 373 participants enrolled in START were infected within 6 months of randomization. Compared to those not recently infected, these participants were younger, had higher HIV RNA levels and were more likely to come from high income countries and from populations such as MSM with more regular HIV testing.

Keywords Self-report · Biomarkers · Recent HIV infection · START trial

Introduction

Self-report of the duration of HIV infection is an important measure for HIV research. Individuals with recent infection can be treated early in their infection, which may limit the size of their viral reservoir [1] and enhance CD4+ recovery [2, 3]. Additionally, newly diagnosed individuals who are recently infected are more likely to be linked to HIV infected individuals who do not know their infection status [4]. Thus, identifying recently infected individuals and then determining their social network and testing those individuals for HIV can decrease the number of HIV infected people unaware of their infection status [4]. Furthermore, if these individuals reduce their risky behaviors as a result of knowing that they are HIV positive, the rate of HIV transmission would be reduced. For these reasons accurate self-report of newly diagnosed individuals is critical for HIV clinical trials [5] and contact tracing [6]. However, the accuracy of self-reported recent HIV infection is not known.

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✉ Oliver Laeyendecker
olaeyen1@jhmi.edu

Extended author information available on the last page of the article

There are many reasons why accurate self-report of recent infection would be useful both scientifically and for public health. Individuals with recent infection have smaller viral reservoirs [1] and are being enrolled for cure protocol studies (Research In Viral Eradication of HIV Reservoirs, protocol # ISRCTN83717528), and prioritized for public health follow-up and partner notification. When using biomarkers to determine recent infection, there are multiple confounding factors that impact their ability to correctly discriminate the duration of infection at the individual level and correctly estimate population level incidence. These include race [7], gender [8], infecting subtype [9–11], set point viral load [12, 13], AIDS [14, 15], viral suppression [13, 14, 16], and viral breakthrough [17]. A multi assay algorithm (MAA) uses a combination of biomarkers with orthogonal properties for misclassification to help maximize the precision in distinguishing recent from non-recent infection [18]. This method has been demonstrated to accurately estimate population level incidence and to accurately identify recent infection at the individual level [19, 20].

We applied this methodology to a subset of participants in the Strategic Timing of AntiRetroviral Treatment (START) trial [21] who were: (1) diagnosed with HIV within 6 months before randomization and also reported being infected within the 6 months before randomization, (2) diagnosed with HIV within 6 months before randomization and did not know their date of infection, and (3) were diagnosed ≥ 2 years before randomization (controls). Our goal was to validate self-report of recency of HIV infection and to use the MAA in combination with self-reported date of infection and documented date of diagnosis to estimate the number of participants enrolled in START with early infection (< 6 months prior to randomization). By identifying a cohort of recently infected individuals randomized to immediate or deferred treatment who will be in long term follow up until 2021, we will be able to determine the health benefits of treatment early in infection.

Methods

START is a multinational randomized clinical trial comparing immediate versus deferred antiretroviral treatment (ART) initiation for major clinical outcomes. The study enrolled 4684 HIV-positive ART-naïve individuals from 35 countries, between April 2009 and December 2013. Participants had to have two CD4 counts > 500 cells/mm^3 at least 2 weeks apart within the 60 days before enrollment and be at least 18 years of age [21]. The study results were unblinded in May 2015 due to clear and significant clinical benefit of immediate ART initiation [21].

At study entry, in addition to demographics, CD4+ count, HIV RNA level and likely mode of HIV infection, two dates were recorded: (1) the date the participant was first diagnosed with HIV as documented in the participant's record; and (2) if known, the month and year the participant believes he/she was first infected with HIV. Plasma samples were collected at entry for future research purposes and stored centrally. These stored samples were tested with the MAA for three groups of participants. Group 1 included 167 participants who were diagnosed and reported being infected ≤ 6 months prior to randomization. Group 2 included 771 participants who were diagnosed ≤ 6 months prior to randomization and who reported the date of infection was unknown. Those diagnosed with HIV between 6 and 24 months of randomization were not studied (n = 1551), as well as those who were diagnosed within 6 months but reported being infected for > 6 months (n = 518). Group 3 consisted of 199 randomly chosen participants who were diagnosed with HIV ≥ 2 years prior to randomization (controls). All samples tested were from individuals who consented to and had stored plasma samples.

Baseline plasma samples were tested by the MAA, which was developed for cross-sectional incidence testing [19]. All samples were tested with the Limiting Antigen-Avidity assay (LAg-Avidity) [22] and those with a LAg-Avidity normalized optical density < 3.0 were further tested by the Johns Hopkins avidity modified GS HIV-1/HIV-2 Plus O EIA [23]. As all participants had a CD4+ count > 500 cells/mm^3 at enrollment, those who had a LAg-Avidity normalized optical density < 2.9, had an avidity index below 85% and a baseline viral load > 400 copies/mL were classified as recently infected. This testing algorithm had been validated in samples from the U.S. where the average duration that an individual appeared recently infected was 146 days [19] and no sample from an individual infected > 4 years was classified as recent. For the algorithm used, it was previously determined that for individuals classified as recent by this testing methodology, 95% were infected < 1 year [19].

Baseline characteristics of Groups 1–3 were summarized with simple summary statistics. The following baseline characteristics were compared: age, gender, race, geographic location, likely mode of HIV infection, baseline CD4+ and CD8+ cell count, and baseline HIV RNA level. These baseline characteristics were chosen to describe the groups because we hypothesized that those with recent infection would differ from those who were not recently infected on many of the characteristics. Specifically, those with recent infection were hypothesized to be younger, men who have sex with men, and enrolled by sites in high-income countries where they were more likely be tested. Those with early infection would also be more likely to have elevated HIV RNA levels. Proportions were compared using Chi squared tests and medians compared using Wilcoxon rank sum tests. Logistic regression analysis which considered multiple factors was used for three comparisons performed in the

following order: (1) within group 1, participants classified as recently infected by the MAA versus those who were not; (2) participants classified as recently infected by the MAA in Group 2 versus all participants in Group 1; and (3) all participants in Group 1 plus participants in Group 2 who were classified as recently infected by the MAA versus participants in Group 2 who were not classified as recently infected by the MAA. Odds ratios were used to compare groups. The first comparison was made in order to determine whether those who self-reported recent infection differed according to the MAA result. The 2nd comparison was made to determine whether those classified as recently infected by the MAA who did not know the date of their infection differed from those who self-reported recent infection. The 3rd comparison was made to describe the differences in baseline characteristics between those we classified as recently infected by self-report or MAA with those who were not classified as recently infected. Statistical analyses were performed using SAS, version 9.3 (SAS Institute, Cary, NC). A p value < 0.05 was considered significant. All p values cited are two-sided and not adjusted for multiple comparisons.

Results

Sample Description and MAA Results

Among the 180 participants who reported being infected within 6 months of randomization, 167 (93%) consented to storage of specimens for future HIV research and had a baseline specimen collected. Of those diagnosed with HIV in the 6 months prior to randomization with unknown date of infection, 93% (771/830) had baseline samples for analysis. 1605 participants reported a diagnosis of HIV two or more years before randomization, 1539 (96%) consented to store specimens, and 199 of those participants (13%) were randomly chosen for this study as controls. Characteristics of the three groups studied are shown in Supplementary Table 1, overall and according to MAA results.

A significantly higher percentage of individuals who reported being infected ≤ 6 months were recently infected by the MAA compared to controls, participants who were diagnosed with HIV for at least 2 years before enrolling in START (65% [109/167] vs. 2.5% [5/199]; χ^2 (1, N = 366) = 166.8, p < 0.001) (Table 1). Additionally, the group who reported being infected ≤ 6 months had lower LAg-Avidity values than the group diagnosed 2 or more years before enrollment (median normalized optical density 1.86 [IQR 0.99, 3.01] vs. 4.53 [3.84, 4.99]; χ^2(1, N = 366) = 162.5, p < 0.001).

Among the 771 participants who were diagnosed with HIV within 6 months prior to enrollment and reported the date of infection as unknown, 206 (26.7%) were recently infected by the MAA. The median LAg-Avidity results for those 206 participants was slightly higher than for the 109 participants in Group 1 who were recently infected by the MAA (OD-n 1.62 [1.05, 2.16] versus 1.46 [0.70, 1.94]; χ^2(1, N = 314) = 4.5, p = 0.034).

Comparison of Baseline Characteristics of Participants Recently Infected Versus not Recently Infected

Among participants in Group 1 (diagnosed with HIV within the past 6 months and HIV infected within the past 6 months) the only baseline characteristic that differed between those classified as recently infected by the MAA and those who were not was HIV RNA level (26,323 copies/mL vs. 19,114 copies/mL; χ^2(1, N = 167) = 2.6, p = 0.11 in univariate analysis and χ^2(1, N = 165) = 4.8, p = 0.028 in multivariate analysis) (Supplementary Tables 1 and 2).

Those in Group 2 (diagnosed with HIV within the past 6 months and with a date of HIV infection unknown) classified as recently infected by the MAA were similar to participants in Group 1 with two exceptions, the percentage

Table 1 Results from the MAA for the three HIV infection groups

	HIV diagnosis date ≤ 6 months		HIV diagnosis date ≥ 2 years (group 3)
	HIV infection date ≤ 6 months (group 1)	HIV infection date unknown (group 2)	
No. tested	167	771	199
Months between diagnosis and enrollment, median [IQR][a]	2.4 [1.8, 3.1]	2.9 [2.1, 4.2]	55.1 [36.7, 85.2]
MAA positive[b], n (%)	109 (65.3)	206 (26.7)	5 (2.5)
LAg-Avidity OD-n[c], median [IQR]	1.86 [0.99, 3.01]	3.46 [2.14, 4.42]	4.53 [3.84, 4.99]

[a] *IQR* interquartile range

[b] *MAA* multi algorithm assay

[c] *OD-n* normalized optical density

of participants who were MSM (69.4 vs. 82.6%; $\chi^2(1, N = 373) = 7.6$, $p = 0.006$ in univariate analysis and $\chi^2(1, N = 373) = 4.3$, $p = 0.039$ in multivariate analysis) and CD8+ count (945 vs. 1,086 cells/mm^3; $\chi^2(1, N = 373) = 7.3$, $p = 0.007$ in univariate analysis and $\chi^2(1, N = 371) = 10.5$, $p = 0.001$ in multivariate analysis, Table 2 and Supplementary Table 1).

Table 3 (and Supplementary Table 3) compares participants in Group 1 together with those in Group 2 who were classified as recently infected by the MAA (recently infected group) to participants in Group 2 who were not classified as recently infected by the MAA. There were a number of differences between these two groups. In the multivariate analysis, participants recently infected by self-report and/or MAA were younger, more likely to be Asian, less likely to black, less likely to be heterosexual, more likely to be enrolled at sites in the U.S., Europe or Australia, and have higher HIV RNA levels.

Discussion

In START we demonstrated that nearly two-thirds of participants who were diagnosed with HIV within 6 months before enrollment and reported HIV infection within 6 months before enrollment had MAA findings which confirmed their recent infection, whereas, only 2.5% of participants who were diagnosed with HIV more than 2 years before enrollment (controls) had positive assay results. Those reporting recent infection and confirmed by the assay did not differ in terms of baseline characteristics from those who were not. This observation taken together with the limitations of the MAA assay used [19], indicate that the classification of participants as recently infected by self-report in START (Group 1) is likely accurate.

There are several limitations to this study. The gold standard used to identify/confirm recent infection was the combination of biomarkers as part of a MAA. The MAA used has a window period of 146 days for HIV-1 clade B infected individuals [19, 26]. This means that the average duration an individual will appear as recently infected by this MAA is 4.8 months (146 days, 95% CI 122 to 170 days) after seroconversion. In START, the median time from diagnosis to

Table 2 Comparison of participants in Group 2 confirmed as recently infected by MAA (unknown HIV infection date, n = 206) with those in Group 1 (self-reported HIV infection ≤ 6 months, n = 167); all participants diagnosed with HIV ≤ 6 months before randomization

	Univariate models[a]			Multivariate model[b]		
	OR [95% CI]	χ^{2c}	p value	OR [95% CI]	χ^{2c}	p value
Age, per 5 years	1.11 [1.00, 1.23]	3.55	0.06	1.06 [0.94, 1.19]	0.86	0.35
Female (ref = male)	1.86 [0.93, 3.72]	3.10	0.08	0.97 [0.36, 2.61]	0.003	0.96
Race/ethnicity (ref = white)		2.23	0.69		0.94	0.92
Asian	1.37 [0.73, 2.56]	0.96	0.33	1.22 [0.59, 2.54]	0.29	0.59
Black	1.24 [0.67, 2.29]	0.48	0.49	0.96 [0.47, 1.95]	0.01	0.91
Latino/hispanic	0.98 [0.54, 1.77]	0.004	0.95	1.01 [0.53, 1.91]	0.0005	0.98
Other	0.68 [0.26, 1.81]	0.59	0.44	0.72 [0.26, 1.97]	0.42	0.52
Likely mode of HIV infection (ref = heterosexual)		8.59	0.014		6.15	0.046
MSM	0.45 [0.26, 0.80]	7.56	0.006	0.43 [0.19, 0.96]	4.25	0.039
Other	0.82 [0.30, 2.23]	0.15	0.70	1.16 [0.37, 3.65]	0.07	0.80
High income region[d] (ref = low-middle income)	0.91 [0.60, 1.37]	0.22	0.64	1.01 [0.62, 1.65]	0.003	0.96
CD4 cell count (per 100 cells/mm^3)	0.99 [0.86, 1.13]	0.05	0.83	1.03 [0.88, 1.20]	0.11	0.74
CD8 cell count (per 100 cells/mm^3)	0.94 [0.91, 0.98]	7.32	0.007	0.93 [0.88, 0.97]	10.47	0.001
HIV RNA ($\log_{10}$ copies/mL)	1.11 [0.83, 1.49]	0.54	0.46	1.34 [0.96, 1.87]	3.03	0.08
No. MAA confirmed	206			206		
Total pts	373			371		

CI confidence interval, *dF* degree(s) of freedom, *MAA* multi algorithm assay, *MSM* men having sex with men, *OR* odds ratio

[a]Odds ratios from individual logistic regression models

[b]Odds ratios from one multivariate logistic regression model with all listed variables. Model limited to participants with complete data

[c]χ^2 tests for univariate models are 1dF, N = 373 except for the omnibus race (4dF, N = 373) and likely mode of HIV infection (2dF, N = 373) tests. Multivariate model significance tests are based on the same dF with N = 371

[d]High income region included the United States, Europe, and Australia. Low-middle includes Latin America, Asia, and Africa

Table 3 Comparison of participants in Group 1 (self-reported HIV infection ≤ 6 months, n = 167) combined with those in Group 2 confirmed as recently infected by the MAA (unknown HIV infection date, n = 206) versus those in Group 2 not confirmed as recently infected (n = 565); all participants diagnosed with HIV ≤ 6 months before randomization

	Univariate models[a]			Multivariate model[b]		
	OR [95% CI]	χ^{2c}	p value	OR [95% CI]	χ^{2c}	p value
Age, per 5 years	0.88 [0.82, 0.95]	12.65	< 0.001	0.90 [0.83, 0.97]	7.36	0.007
Female (ref = male)	0.26 [0.18, 0.37]	51.79	< 0.001	0.75 [0.44, 1.27]	1.15	0.28
Race/ethnicity (ref = white)		68.40	< 0.001		31.94	< 0.001
Asian	1.72 [1.07, 2.77]	5.04	0.024	3.14 [1.78, 5.53]	15.69	< 0.001
Black	0.28 [0.20, 0.40]	49.44	< 0.001	0.63 [0.41, 0.98]	4.29	0.038
Latino/hispanic	1.04 [0.69, 1.56]	0.03	0.86	1.26 [0.81, 1.99]	1.04	0.31
Other	0.58 [0.32, 1.06]	3.18	0.08	0.78 [0.41, 1.50]	0.55	0.46
Likely mode of HIV infection (ref = heterosexual)		76.69	< 0.001		13.12	0.001
MSM	4.06 [2.97, 5.55]	76.67	< 0.001	2.31 [1.44, 3.70]	11.99	< 0.001
Other	2.60 [1.44, 4.70]	10.00	0.002	2.32 [1.17, 4.59]	5.81	0.016
High income region[d] (ref = low-middle income)	2.14 [1.63, 2.82]	29.49	< 0.001	1.62 [1.15, 2.28]	7.58	0.006
CD4 cell count (per 100 cells/mm^3)	0.92 [0.85, 0.99]	4.81	0.028	1.08 [0.99, 1.19]	3.00	0.08
CD8 cell count (per 100 cells/mm^3)	1.01 [0.98, 1.04]	0.50	0.48	0.98 [0.95, 1.01]	1.44	0.23
HIV RNA ($\log_{10}$ copies/mL)	1.99 [1.68, 2.36]	63.30	< 0.001	1.83 [1.50, 2.22]	36.38	< 0.001
No. recently infected (MAA or self-report)	373			371		
Total pts	938			931		

CI confidence interval, *dF* degree(s) of freedom, *MAA* multi algorithm assay, *MSM* men having sex with men, *OR* odds ratio

[a]Odds ratios from individual logistic regression models

[b]Odds ratios from one multivariate logistic regression model with all listed variables. Model limited to participants with complete data

[c]χ^2 tests for univariate models are 1dF, N = 938 except for the omnibus race (4dF, N = 938) and likely mode of HIV infection (2dF, N = 938) tests. Multivariate model significance tests are based on the same dF with N = 931

[d]High income region included the United States, Europe, and Australia. Low-middle includes Latin America, Asia, and Africa

enrollment was 2.4 months for those who reported being infected ≤ 6 months. MAA was based on a plasma sample taken at the time of randomization. Had a plasma sample been available at the time of HIV diagnosis to study the agreement of the MAA and self-reported recent infection, agreement would likely have been higher. Furthermore, of the five individuals who were known to be infected more than 2 years, but were classified as recently infected by the MAA, three were subjects where the time between diagnosis and blood draw was < 4 years. Additionally, four of these five individuals were from countries where HIV-1 non-B clades circulate [24]. Since the incidence assays used have different properties depending on the infecting clade [10, 11, 22], it is not surprising that the majority of the misclassified long term infected samples come from HIV-1 non-B endemic areas.

Among participants with an unknown infection date who were diagnosed with HIV in the 6 months prior to enrollment, 206 (27%) were recently infected according to the MAA. These 206 participants had similar baseline characteristics to those who self-reported being recently infected. Thus, taken together with those self-reporting recent infection, 373 participants in START were infected within 6 months before enrollment. Compared to the 565 participants diagnosed with HIV in the 6 months prior to enrollment who were not recently infected, the 373 participants who were recently infected were younger, more likely to be Asian, less likely to black, less likely to be heterosexual, more likely to be enrolled at sites in the U.S., Europe or Australia, and have higher HIV RNA levels.

There are several explanations for why particular factors were associated with recent infection in the START trial population. The entry requirements for the trial required high CD4 counts which would select for individuals who were early in their infection or with a less pathogenic strain of HIV. Therefore the finding that higher viral load was associated with recent infection is not surprising. The majority of heterosexual participants in the trial came from Africa, where frequent testing is not the norm. For example a recent study in Zambia, 40% of men had never been tested for HIV in their lifetime [25]. The increased odds of Asians being recently infected is not surprising as most of those subjects came from Thailand which has a long history of HIV prevention and 75% of MSM reporting getting tested for HIV in the past year [25].

Although we do not know of any published studies comparing self-report of recency of HIV infection to biomarkers, a few studies have compared self-report of HIV status to biomarkers of HIV infection. For example, Fisher et al. compared enzyme-linked immunosorbent assay test results to self-report of HIV status, finding that the specificity of self-report was above 99% at baseline and follow-up while the sensitivity was 32% at baseline and 61% at follow-up [26]. Additionally, Ross et al. compared Western blot results to self-reported HIV infection and found that the sensitivity of self-report was 93% and the specificity was above 99% [27]. However in our study there were no pre-determined reasons for misinforming the study staff about their date of HIV infection. Our results showed a similarly high level of self-report specificity compared to these two studies while our sensitivity was lower than that observed by Ross et al. and slightly higher than the sensitivity seen by Fisher et al. at follow-up [26, 27].

In summary, in the START trial 373 participants with recent HIV infection were enrolled. To our knowledge, this subgroup represents the largest study of immediate versus deferred ART among individuals recently infected with HIV.

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Compliance with Ethical Standards

Conflict of interest All the authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

Research Involving human and Animal Participants This article does not contain any studies with animals performed by any of the authors.

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Affiliations

Katherine E. Schlusser[1] · Shweta Sharma[2] · Pola de la Torre[3] · Giuseppe Tambussi[4] · Rika Draenert[5] · Angie N. Pinto[6] · Julia A. Metcalf[7] · Danielle German[8] · James D. Neaton[2] · Oliver Laeyendecker[1,9] · for the INSIGHT START Study Group

Katherine E. Schlusser
kschlus1@jhmi.edu

Shweta Sharma
shwetas@ccbr.umn.edu

Pola de la Torre
delaTorre-Pola@CooperHealth.edu

Giuseppe Tambussi
tambussi.giuseppe@hsr.it

Rika Draenert
Rika.Draenert@med.uni-muenchen.de

Angie N. Pinto
apinto@kirby.unsw.edu.au

Julia A. Metcalf
jmetcalf@niaid.nih.gov

Danielle German
dgerman1@jh.edu

James D. Neaton
neato001@umn.edu

[1] Department of Medicine, Johns Hopkins University School of Medicine, 855 North Wolfe St., Rangos Building, room 538A, Baltimore, MD 21205, USA

[2] Division of Biostatistics, School of Public Health, University of Minnesota, Minneapolis, MN, USA

[3] Cooper University Hospital, Camden, NJ, USA

[4] IRCCS-Ospedale San Raffaele, Milan, Italy

[5] Section Clinical Infectious Diseases, Klinikum der Universität Munich, Medizinische Klinik IV, Munich, Germany

[6] The Kirby Institute, UNSW Australia, Sydney, Australia

[7] Division of Clinical Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

[8] Department of Health, Behavior and Society, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA

[9] Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA