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2012年11月

G. 知的所有権の出願・取得状況（予定を含む）

1. 特許取得

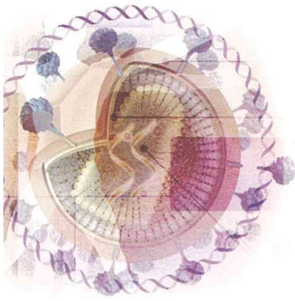
なし

2. 実用新案登録

なし

3. その他

なし



多施設による急性期患者病状進行解析研究

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研究要旨

HIV感染症の病状進行を評価する目的で、感染初期にHIV感染が診断され患者を対象とする多施設共同コホート研究（East Asia Clinical HIV Cohort: EACH Cohort）を設立した。H23年度に単施設でデータ収集が開始され、H24年度には国内4施設・国外1施設と参加施設が拡大した。集められたデータを公平に利用するシステムを構築するため、運営規則やマニュアルを整備した。EACH Cohortは日本初の多施設共同HIVコホートであり、今後もコホートの拡大を目指す。

A. 研究目的

世界のHIV治療ガイドラインは、欧米人を対象にした研究をもとにして作られてきたが、近年人種の違いが副作用や血中濃度等に影響することが分かってきた。本研究は、それらの知見を検証し本邦における最適な治療ガイドラインを作成するため、多施設共同の患者コホートEast Asia Clinical HIV Cohort（EACH Cohort）を設立し臨床研究基盤を整備することを目標としている。

最近ウイルス自体が宿主の免疫機構から効率的に逃避する変異を獲得し、病状を早く進行させるような進化を遂げたことが明らかになりつつある[1,2]。病状の進行とHLAには相関があり、特にHLA-B*5101などは病状の進行を遅らせることで知られているが、日本ではHLA-B*51拘束性のCTLから逃避したウイルスが蓄積し、初感染から3年程度で治療を必要とするレベルにまで病状が進行し、HLA-B*51を持つ患者ほど病状の進行が早くなっていることが分かった[1-3]。CTLの逃避と病状の進行の関連を示した報告はまだ無く、このことが普遍的であればHIV感染症の病態を考える上で非常に重要な知見となる。また、このようなHIVに対する特異免疫やウイルスの免疫逃避機構の研究はワクチン開発に不可欠であるが、免疫能がHIVによって破壊されたりHIVが体内で変異を獲得したりする以前、つまり感染早期の段階での研究介入・評価が必要となる。臨床疫学的にも、HIVに感染しても数年は無症状で感染に気付かないため、感染を起点にした予後の正確な評価のため感染早期と判定された症例を対象にした研究が求められている。このような背景をふまえ、コホート研究の最初の対象者を早期HIV感染症例と設定した。

B. 研究方法

①研究デザイン

HIV感染者を対象としたコホート研究である。

②対象

対象は、「早期HIV感染者」で、6ヶ月以内に以下の（a），（b），（c）いずれかが認められた者とした。

(a) HIV 抗体陰性かつ HIV-RNA 陽性
(b) WB における経時的な陽性バンドの増加
(c) 12 ヶ月以内の HIV 抗体陽転化

③評価項目および Endpoint

CD4 数、ウイルス量、合併症、治療内容等の臨床データを、診断から 1、2、3、6、12 ヶ月後、以降 6 ヶ月毎に収集する。感染から ART 開始もしくは CD4 数 350/mm^2 未満に達するまでの期間を primary endpoint とし、その他、エイズ発症までの期間、非エイズ関連合併症を発症するまでの期間につき、Kaplan-Meier 法で解析を行うとともに、CD4 の早期低下に寄与する背景因子について、多変量解析を行う。

目標例数は、過去の症例数をもとに、十分に記述統計解析が実施できる例数を設定した。すなわち、1997 年から 2007 年の 10 年間に、国立国際医療研究センターエイズ治療研究開発センターで経験した急性 HIV 感染者は 108 例で、年間平均 11 例である。感染早期は、他院への転院や、追跡不能となる症例が比較的少なく年間 0-1 例である。よって、本試験における前向きの患者登録は、5 年間で 50 例と推計した。また、今年度、過去に早期 HIV 感染の診断時期が特定できる症例を新たに加えるよう、研究計画を修正し、登録患者数の増加を図った。

(倫理面への配慮)

すべての施設で倫理委員会の承認を得ることを前提とし、「臨床試験に関する倫理指針」(平成 15 年 7 月 30 日制定、平成 20 年 7 月 31 日全部改正、厚生労働省) および「疫学研究に関する倫理指針 (平成 14 年 6 月 17 日制定、平成 19 年 8 月 16 日全部改正、平成 20 年 12 月 1 日一部改正) 文部科学省、厚生労働省」に準拠して実施する。

主たる研究施設である国立国際医療研究センターにおいて 2009 年 10 月 1 日に倫理委員会からの承認を得た。その後、2011 年 9 月 15 日に後ろ向きのデータ収集、検体保存、多施設化に関する内容が加えられた (承認番号 NCGM-G-000733-02)。

C. 研究結果

①参加施設

今年度、新たに国内 2 施設、国外 1 施設で倫理委員会の承認が得られ、参加施設は 5 施設 (国立国際医療研究センター、国立病院機構九州医療センター、北海道大学病院、順天堂大学医学部附属順天堂医院、韓国ヨンセイ大学医学部) となった。シンガポールの Tan Tock Seng 病院からも EACH Cohort 参加の意向が届いており、国内施設の参加が進まない一方で、これらアジアの代表的な HIV 診療施設から積極的な共同研究の要請があることから、国際展開を前提とした軌道修正が必要と考えられた。そのため、今年度は研究計画書と運営規則の改訂についても協議を進め、1) 疫学・統計の各専門家をアドバイザーとしてチームに加えること、2) 英語のプロトコールを基本とすること、3) 検体バンクは国内だけで展開することなど、来年度以降の重要な方針を決定した。

②患者登録・追跡状況

韓国ヨンセイ大学の 15 例を含む 268 例の登録例の内訳を表 1 に示す。平成 25 年 2 月末現在、すでに 4 例の死亡が確認され、188 例が観察を継続している。患者追跡率を図 1 に示す。受診中断による追跡不能例 (lost to follow) の発生は 1.91/100 人年であった。

③登録患者背景

ベースラインのデータ収集が完了している国内 4 施設の 253 例について、その患者背景を表 2 に示す。男性が 240 例 (95%)、年齢中央値が 32 歳 (IQR 26-38) であった。死亡 4 例の死因は、3 例が自殺、1 例が水難事故であった。

表 1　平成 25 年 2 月 28 日現在の EACH Cohort 登録状況

Status	n	%
全登録数	268	100.0
観察中	188	70.1
観察終了 (死亡)	4	1.5
受診中断による追跡不能	27	10.1
転院による追跡不能	49	18.3

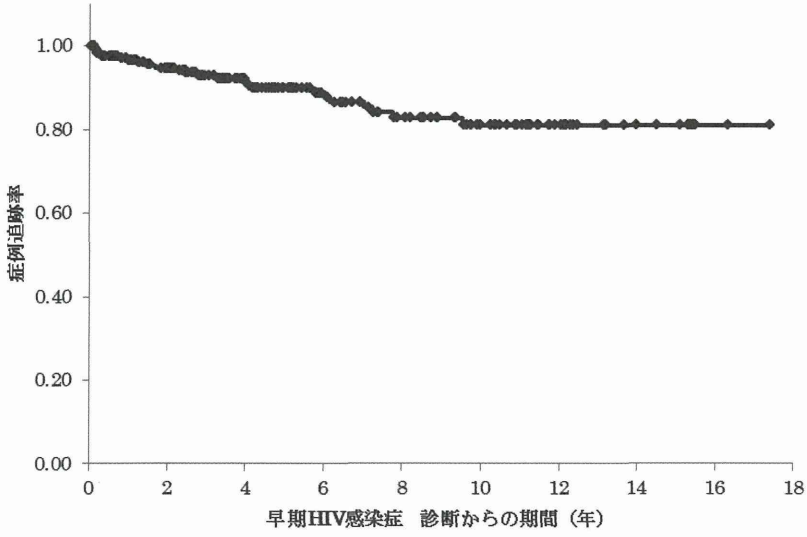

図 1　EACH Cohort における症例追跡維持率
（早期 HIV 診断から受診中断による追跡不能までの期間。死亡・転院は打ち切りとする。）

表 2　EACH Cohort 患者背景（平成 25 年 2 月 28 日現在）

		n=253
男性, % (n)	95	(240)
年齢, median years (IQR)	32	(26-38)
HIV 感染経路, % (n)		
Homosexual	81.8	(207)
Heterosexual	7.5	(19)
Bisexual	7.9	(20)
Injection drug use	0.8	(2)
その他/不明	2.0	(4)
CD4 数, median /mm^3 (IQR)	345	(230-486)
HIV-RNA 量　median log copies/ml (IQR)	5.3	(4.6-6.0)
HBs 抗原陽性, % (n)	2.9	(7/242)
HCV 抗体陽性, % (n)	1.7	(4/241)
診断年, % (n)		
1997 以前	5.5	(14)
1998-2000	6.7	(17)
2001-2003	17.0	(43)
2004-2006	11.1	(28)
2007-2009	27.7	(70)
2010-2012	31.6	(80)

④登録患者における治療開始までの期間

同じく国内 4 施設の 253 例において、早期 HIV 感染症診断から治療開始までの期間は平均 2.57 年であった。未治療者の割合の変化を図 2 に示す。今後、未収集のデータが集まるのを待って、未治療時の CD4 数の低下速度（病状進行速度）とそれに影響する因子について、より詳しい解析を行う予定である。

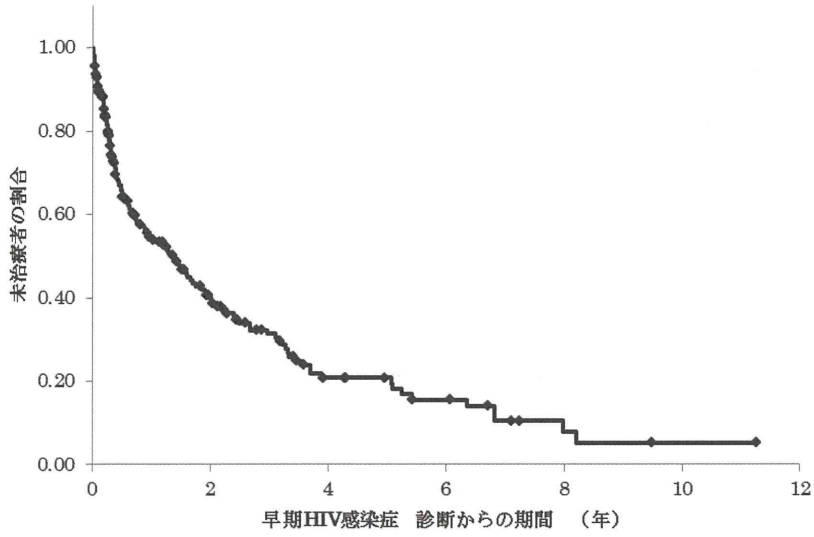

図２　EACH Cohort 内の未治療者の割合
早期 HIV 感染（1 年以内の感染）診断から抗 HIV 療法開始までの期間（Structured Treatment Interruption ： STI 症例を除く）

D. 考察

多施設コホート研究は HIV 研究における基本形態のひとつと位置づけられているが、日本にはこれまで存在しなかったため、EACH Cohort に寄せられる期待は大きい。しかし、韓国やシンガポールといったアジア諸国との連携を達成する一方で、データ収集にかかるマンパワー不足が参加の障壁となって国内施設の参加は 4 施設に止まった。今後は、臨床研究コーディネーターの育成や、疫学や統計の専門家らとの連携に力を入れる必要がある。

E. 結論

国内初の多施設共同 HIV 感染者コホート研究において、早期 HIV 感染者を対象にデータベースの開発が完了し、データ収集が開始された。コホートの維持・発展のため、人的支援や公平で透明性の高いデータ利用システムを作ることが必要である。

F. 研究発表

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なし

2. 実用新案登録

なし

3. その他

なし

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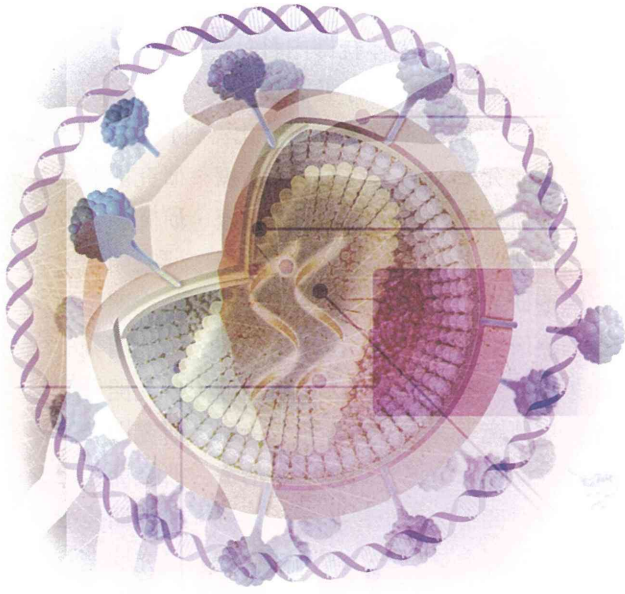

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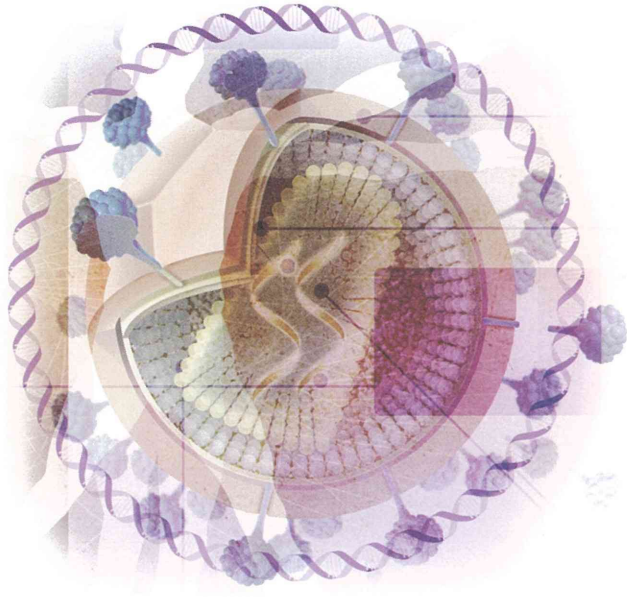
研究成果の刊行に関する一覧



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Trends in early and late diagnosis of HIV-1 infections in Tokyoites from 2002 to 2010

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SUMMARY

Objective: The objective of this study was to delineate the trends in early and late diagnosis of HIV-1 infection in newly diagnosed Tokyoites.
Methods: The BED assay was used to identify cases diagnosed at an early stage of infection. BED-positive non-AIDS cases with a CD4 cell count ≥200/μl were defined as cases with recent infection. The rates of AIDS and recent infection in 809 newly diagnosed Tokyoites during 2002–2010 were analyzed.
Results: The AIDS rate was 22.5%. AIDS patients were older (40.4 years) than non-AIDS patients (35.0 years), and a smaller proportion were men who have sex with men (MSM) in AIDS patients (81.7%) than in non-AIDS patients (89.9%). The AIDS rate was persistently lower (≤14.3%) in ≤29-year-old than in ≥30-year-old MSM. The rate of recent infection was 24.4%. Individuals with recent infection (33.0 years old) were younger than the others (37.2 years). The rate of recent infection was lower (≤18.5%) in MSM aged ≥40 years than in those aged ≤39 years during the study period, except for 2007 and 2008.
Conclusions: Younger MSM Tokyoites appear to be aware of the risk of their sexual behavior, sufficient to take voluntary HIV testing repeatedly, resulting in early diagnosis. Older MSM did not take HIV testing frequently enough and may be a good target for campaigns promoting testing.

1. Introduction

The overall growth of the global AIDS epidemic appears to have stabilized. The annual number of new cases of HIV infection has been in steady decline since the late 1990s.[1] In Japan, however, the annual number of newly diagnosed cases has almost doubled during the most recent decade (791 cases in 2000 and 1544 cases in 2010), although the prevalence of HIV in the adult population remains <0.1%.[2] The distribution of these cases is heavily concentrated in large cities, and approximately 35% of the newly diagnosed cases have been identified in Tokyo.[3]

Early diagnosis of HIV infection is critically important because some AIDS-defining diseases are fatal, even in the era of combination antiretroviral treatment (ART); also the introduction of ART after the development of AIDS is often complicated with immune reconstitution inflammatory syndrome (IRIS).[4,5] In this regard, the introduction of ART at the early stages seems to significantly reduce the sexual transmission of HIV-1.[6,7] Thus, it is important to identify newly infected individuals and provide early ART to reduce the incidence of AIDS and transmission of HIV. Knowledge about the proportion of patients diagnosed at the early stage of an HIV infection in the newly diagnosed cases is also useful for planning and evaluation of any prevention program and for resource allocation.[8,9] However, it is usually difficult to distinguish recent from long-standing HIV infections except for acute symptomatic infections.[10] Simple prediction of the infection time from CD4 cell counts appears inaccurate because the disease progression rate varies enormously among infected individuals.[11] The BED HIV-1 capture enzyme immunoassay (BED assay) uses the branched peptide to detect HIV-1 IgG antibodies from all subtypes (i.e., HIV-1 B, E, and D gp41 immunodominant sequences are included on a branched peptide used in the assay) and measures levels of anti-HIV-1 IgG relative to total IgG.[12] Since the ratio of anti-HIV-1 IgG to total IgG increases with time shortly after HIV-1 infection, the HIV-1-infected patient is considered to have recently acquired the infection when the normalized optical density (ODn) is less than 0.8 on the BED assay (ODn reaches 0.8 on average 197 days after seroconversion.).[13]

The present study was an attempt to delineate the trends in early diagnosis of HIV-1 infection in Tokyo from 2002 to 2010 by using the BED assay. The aim of this analysis was to enhance our understanding of the status of HIV-1 spread in Tokyo and to help in the design of strategies to control the HIV-1 epidemic in Japan.

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2. Materials and methods

2.1. Newly diagnosed patients

This study included all ART-naïve HIV-1-infected individuals who met the following criteria: (1) those who visited the AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, between 2002 and 2010 within 30 days of their diagnosis with an HIV-1 infection and (2) availability of plasma samples taken at the first visit under signed informed consent for use in viral, immunological, and epidemiological studies. Participant information including CD4 count, HIV-1 load, age at the first visit, gender, nationality, probable HIV-1 transmission route, and history of HIV testing, were collected from the medical records. According to the Japanese law for infection control, physicians are obliged to report newly diagnosed HIV/AIDS cases to the National AIDS Surveillance Committee (the Ministry of Health, Labor, and Welfare of the Japanese Government). A total of 11 673 HIV/AIDS cases nationally, including 4048 cases diagnosed in Tokyo (Tokyo cases), which were entered into the registry of this committee from 2002 to 2010, were used as the control populations to evaluate the representativeness of the patients enrolled in the present study (AIDS Clinical Center cases).[2,3] Plasma samples obtained from the participants were stored at −80 °C. The viral subtype in each case was determined from the HIV-1 protease–reverse transcriptase sequence (which was analyzed for drug resistance genotyping) by the neighbor-joining method using the Genetic-Win system (Software Development, Tokyo).[14]

This study was conducted according to the principles of the Declaration of Helsinki and was approved by the ethics committee of the National Center for Global Health and Medicine.

2.2. BED assay

The BED HIV-1 capture enzyme immunoassay (BED assay; Calypte Biomedical Corp., Portland, OR, USA) was used to estimate the time of HIV-1 infection.[12] In accordance with the manufacturer's instructions, 5 μl of plasma was diluted with 500 μl of the diluent in the kit, and the proportion of anti-HIV-1-specific IgG to the total IgG in the sample was measured by optical density (OD). The OD values of the test specimens were normalized (ODn) relative to the value of a calibrator (specimen OD/calibrator OD) to minimize inter-run variation. Samples with ODn ≤0.8 were considered to be from individuals who had seroconverted within 197 days and were defined as BED-positive.[13] BED-positive non-AIDS cases with CD4 cell counts ≥200/μl were defined as individuals with recent infection. The others were defined as chronic infection.

2.3. Statistical analysis

Differences in demographic data including age, gender, risk behavior, nationality, and AIDS development among the AIDS Clinical Center cases, national cases, and Tokyo cases, were examined for significance using one-way analysis of variance (ANOVA) and the Tukey test, or Pearson's Chi-square test. Differences in demographic data including age, CD4 count, logarithmic HIV-1 viral load, nationality, transmission category, HIV-1 subtype, cue for HIV diagnosis, and history of HIV testing, between AIDS and non-AIDS patients and between recent and chronic infection, were examined for significance using the *t*-test or Pearson's Chi-square test. To estimate the correlation with the development of AIDS, binominal logistic regression analysis including age, nationality (Japanese or not), and transmission category (men having sex with men (MSM) or not) was performed. A *p*-value of less than 5% denoted statistical significance. Statistical analyses were performed with SPSS Statistics 17.0 (IBM Japan Inc., Tokyo, Japan) and Stat Mate II (NANKODO, Tokyo).

3. Results

3.1. Newly diagnosed cases of HIV-1 infection

The study subjects were 809 ART-naïve HIV-1-infected patients. All of them had visited the AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, within 30 days of the diagnosis of HIV-1 infection (median 8 days) between 2002 and 2010. They included 741 Japanese, 35 Asians other than Japanese, and 33 from other countries. They represented 20.0% of the total number of newly diagnosed Tokyoite cases during the same period (Table 1). There were no significant differences in the proportion of AIDS (22.5% vs. 21.9%), percentage of males (96.2% vs. 94.3%), or proportion of Japanese (91.6% vs. 90.7%) between our study patients and those of the Tokyo registry, although our patients included a significantly smaller proportion of AIDS cases (22.5% vs. 30.4%) and significantly larger population of male patients (96.2% vs. 91.8%) and Japanese patients (91.6% vs. 88.5%) compared with the patients of the national registry. Furthermore, our patients were significantly younger than the patients of the Tokyo and national registries (36.2 vs. 37.7 and 38.0 years), and the proportion of MSM among male patients was significantly higher than in the Tokyo and national registries (88.0% vs. 72.8% and 59.8%).

Subtype analysis successfully determined the HIV-1 subtype in 807 patients (99.8%); the majority were infected with HIV-1 subtype B (742 patients, 91.9%), while 5.7% were infected with HIV-1 subtype AE, which is comparable to previously published subtype data in Japan.[14] The HIV-1 subtype could not be determined in two patients because the viral load was below the detection limit (<40 copies/ml), although they were not being treated with anti-HIV drugs.

3.2. Features of AIDS patients

Among the 809 cases, 182 (22.5%, 95% confidence interval (95% CI) 19.6–25.4) had already developed AIDS at the first visit, while the other 627 were non-AIDS cases (Table 2). AIDS cases were significantly older (40.4 years, 95% CI 38.8–41.9 vs. 35.0 years, 95% CI 34.2–35.9), and as expected, had lower CD4 counts (61.7/μl, 95% CI 50.6–72.8 vs. 318.0/μl, 95% CI 303.0–333.0) and higher viral loads (5.22 log VL/ml, 95% CI 5.13–5.31 vs. 4.63 log VL/ml, 95% CI 4.56–4.70) than non-AIDS patients. There were no significant differences in nationality (Japanese 91.8%, 95% CI 87.8–95.8 vs. 91.5%, 95% CI 89.4–93.7) or HIV-1 subtype (subtype B 89.0%, 95% CI 84.5–93.6 vs. 92.5%, 95% CI 90.4–94.6) between AIDS and non-AIDS

Table 1
New cases of HIV-1-infected patients diagnosed between 2002 and 2010

	Japan[a]	Tokyo[b]	This study
Number of cases	11 673	4048	809
Age, years (mean ± SD)	38.0 ± 11.8[c]	37.7 ± 11.9[d]	36.2 ± 11.0
Males	10 721 (91.8%)[c]	3819 (94.3%)	778 (96.2%)
Men having sex with men	6408 (59.8%)[c]	2780 (72.8%)[c]	685 (88.0%)
Japanese	10 335 (88.5%)[d]	3673 (90.7%)	741 (91.6%)
AIDS cases	3551 (30.4%)[c]	885 (21.9%)	182 (22.5%)

Statistical analyses were performed by one-way ANOVA and Tukey test, or Chi-square test.
[a] Provided by the National AIDS Surveillance Committee (the Ministry of Health, Labor, and Welfare of the Japanese Government).
[b] Provided by the Bureau of Social Welfare and Public Health, Tokyo.
[c] $p < 0.001$, compared with the study participants.
[d] $p < 0.01$ compared with the study participants.

Table 2
Demographics of participants with and without AIDS

	AIDS (n = 182)		Non-AIDS (n = 627)		p-Value[a]
	Mean	(95% CI)	Mean	(95% CI)	
Age (years)	40.4	(38.8–41.9)	35.0	(34.2–35.9)	<0.001
CD4 count /μl	61.7	(50.6–72.8)	318.0	(303.0–333.0)	<0.001
Log viral load/ml	5.22	(5.13–5.31)	4.63	(4.56–4.70)	<0.001
	n	% (95% CI)	n	% (95% CI)	
Nationality					0.424
Japan	167	91.8 (87.8–95.8)	574	91.5 (89.4–93.7)	
Asia other than Japan	11	6.0 (3.3–10.8)	24	3.8 (2.6–5.7)	
North and South America	2	1.1 (0.2–4.0)	17	2.7 (1.7–4.3)	
Africa	2	1.1 (0.2–4.0)	6	1.0 (0.4–2.1)	
East and West Europe	0	0 (0–2.0)	4	0.6 (0.2–1.6)	
Oceania	0	0 (0–2.0)	2	0.3 (0–1.1)	
Transmission category					
Male	175	96.2 (93.4–98.9)	603	96.2 (94.7–97.7)	0.024
MSM	143	81.7 (76.0–87.4)	542	89.9 (87.5–92.3)	
Heterosexual	21	12.0 (7.2–16.8)	43	7.1 (5.4–9.6)	
IDU	1	0.6 (0–3.2)	2	0.3 (0.1–1.2)	
Unknown	10	5.7 (3.0–10.5)	16	2.7 (1.6–4.3)	
Female	7	3.8 (1.7–7.9)	24	3.8 (2.6–5.7)	-
Heterosexual	7	100 (46.8–100)	24	100 (100–100)	
Subtype					0.351
B	162	89.0 (84.5–93.6)	580	92.5 (90.4–94.6)	
AE	16	8.8 (5.4–14.3)	30	4.8 (3.4–6.8)	
C	1	0.5 (0–3.0)	7	1.1 (0.5–2.3)	
G	2	1.1 (0.2–4.0)	3	0.5 (0.1–1.4)	
AG	1	0.5 (0–3.0)	3	0.5 (0.1–1.4)	
A	0	0 (0–2.0)	2	0.3 (0–1.1)	
Unknown	0	0 (0–2.0)	2	0.3 (0–1.1)	
Cue for HIV diagnosis					<0.001
Voluntary testing	12	6.6 (3.7–11.5)	283	45.1 (41.2–49.0)	
Provider-initiated testing	167	91.8 (87.8–95.8)	338	53.9 (50.0–57.8)	
Unknown	3	1.6 (0.4–4.8)	6	1.0 (0.4–2.1)	
Previous testing					<0.001
Yes	29	15.9 (10.6–21.3)	282	45.0 (41.1–48.9)	
No	65	35.7 (28.8–42.7)	254	40.5 (36.7–44.4)	
Unknown	88	48.4 (41.1–55.6)	91	14.5 (11.8–17.3)	
BED assay					<0.001
Recent (ODn ≤0.8)	47	25.8 (19.5–32.2)	255	40.7 (36.8–44.5)	
Chronic (ODn >0.8)	135	74.2 (67.8–80.5)	372	59.3 (55.5–63.2)	

CI, confidence interval; MSM, men who have sex with men; IDU, intravenous drug user; ODn, normalized optical density.
[a] By t-test or Pearson's Chi-square test.

cases (Pearson's Chi-square test). MSM activity was the most frequent transmission route in both groups, and still more frequent in non-AIDS cases (89.9%, 95% CI 87.5–92.3) than in AIDS cases (81.7%, 95% CI 76.0–87.4). A larger proportion of patients in the non-AIDS group than in the AIDS group had undertaken previous HIV testing (45.0%, 95% CI 41.1–48.9 vs. 15.9%, 95% CI 10.6–21.3) and had been diagnosed with HIV-1 infection by voluntary testing (45.1%, 95% CI 41.2–49.0 vs. 6.6%, 95% CI 3.7–11.5), suggesting that repeated voluntary testing may prevent disease progression to AIDS in the high-risk groups.

Binominal logistic regression analysis of age, nationality (Japanese or not), and transmission category (MSM or not) identified age as the most significant factor associated with the development of AIDS (per 1-year increment, (hazard ratio) HR 1.041, 95% CI 1.026–1.057; $p < 0.001$).

To delineate the trends in late diagnosis of HIV-1 infection, the annual rates of AIDS cases in newly-diagnosed HIV-1-infected patients were plotted through the study period. The rate of AIDS cases remained around 30% between 2002 and 2004. It decreased to 15.0% in 2005, but then showed a gradual increase annually, reaching 24.8% in 2010 (Figure 1). To identify the population that influenced the increase in the rate of AIDS cases in the most recent years, we selected and categorized the study participants based on their features. Specifically, we focused on MSM patients, because 85% of our patients were MSM. Based on the above results of the significance of age in the binominal logistic regression analysis in the development of AIDS, we examined the effect of age in more detail by dividing the MSM patients into three age groups: those aged ≤29 years (217 patients, 31.7%), 30–39 years (273 patients, 39.9%), and ≥40 years (195 patients, 28.5%). In the ≥40 years MSM group, the rate was higher than 50% between 2002 and 2004, but decreased to 21.4% in 2005 and further decreased to 14.3% in 2006, but gradually increased and reached ~30% in 2009 and 2010 (Figure 1). On the other hand, in the ≤29 years MSM group, the AIDS rate was steadily lower than 20%, indicating that most young HIV-1-infected MSM were diagnosed before the development of AIDS throughout the study period. The AIDS rate in the 30–39 years MSM group was between those of the other two groups during most of the study period. A significantly larger proportion of patients in the ≤29 years MSM group had undergone voluntary HIV testing (43.8%, $p = 0.002$, Pearson's Chi-square test) and diagnosis with HIV (48.8%, $p < 0.001$, Pearson's Chi-square test), compared with the 30–39 years MSM group (43.6% and 36.6%, respectively) and the ≥40 years MSM group (34.9% and 32.3%, respectively). These results suggest that repeated voluntary testing may have prevented disease progression to AIDS in the younger MSM groups. The high rate of AIDS in all the study participants observed in 2002–2004 seemed mainly due to the ≥40-year-old MSM. Furthermore, the gradual increase in the AIDS rate in the ≥40-year-old MSM since 2006 also seemed to have contributed to

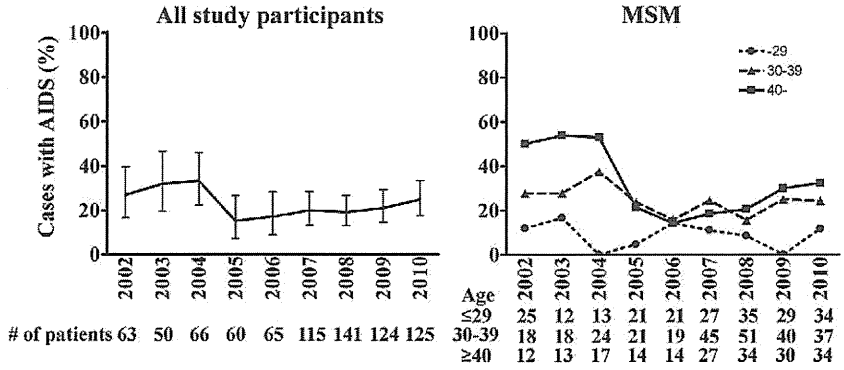

Figure 1. Annual rate of AIDS in newly diagnosed HIV-1-infected individuals. The annual AIDS rate for all study participants (809 patients; left panel), and men who have sex with men (MSM) categorized by age: ≤29 years (n = 217), 30–39 years (n = 273), and ≥40 years (n = 195) (right panel). The 95% confidence intervals are also shown in the left panel. Data including 95% confidence intervals for the MSM are provided in the **Supplementary Information** (Table S1).

the rising AIDS rate in all, suggesting that older MSM should be the main target for interventions aimed at promoting HIV testing for early diagnosis and prevention of the development of AIDS.

3.3. Trends in early HIV diagnosis

To identify individuals with recent HIV-1 infection, we performed a BED assay for the 809 study participants. Before analysis of the results, we dealt with the problem of potential misclassification. Previous studies reported small levels of anti-HIV-1-specific IgG relative to the total IgG in cases with both recent HIV-1 infection and long-standing chronic cases with severe immunodeficiency, which could result in false classification of chronic cases as recent infection.[12,15,16] To tackle this problem, previous studies classified AIDS cases and cases with CD4 cell counts <200/μl as chronic infection cases, in accordance with the Joint United Nations Programme on HIV/AIDS (UNAIDS)/World Health Organization (WHO) guidelines.[17–21] We applied

Table 3
Demographics of participants with recent and chronic infection

	Recent (n = 197)		Chronic (n = 612)		p-Value[a]
	Mean	(95% CI)	Mean	(95% CI)	
Age (years)	33.0	(31.7–34.3)	37.2	(36.3–38.1)	<0.001
CD4 count /μl	423.2	(399.2–447.3)	207.9	(193.3–222.4)	<0.001
Log viral load/ml	4.61	(4.46–4.76)	4.81	(4.74–4.87)	0.005
	n	% (95% CI)	n	% (95% CI)	
Nationality					0.101
Japan	189	95.9 (93.2–98.7)	552	90.2 (87.8–92.6)	
Asia other than Japan	2	1.0 (0.2–3.7)	33	5.4 (3.9–7.6)	
North and South America	3	1.5 (0.4–4.4)	16	2.6 (1.6–4.2)	
Africa	1	0.5 (0–2.8)	7	1.1 (0.5–2.4)	
East and West Europe	1	0.5 (0–2.8)	3	0.5 (0.1–1.4)	
Oceania	1	0.5 (0–2.8)	1	0.2 (0–0.9)	
Transmission category					
Male	192	97.5 (95.3–99.7)	586	95.8 (94.2–97.3)	0.314
MSM	177	92.2 (88.4–96.0)	508	86.7 (83.9–89.4)	
Heterosexual	11	5.7 (3.1–10.2)	53	9.0 (7.0–11.8)	
IDU	0	0 (0–1.9)	3	0.5 (0.1–1.5)	
Unknown	4	2.1 (0.7–5.3)	22	3.8 (2.5–5.7)	
Female	5	2.5 (1.0–5.9)	26	4.2 (2.9–6.2)	-
Heterosexual	5	100 (34.4–100)	26	100 (81.5–100)	
Subtype					0.029
B	188	95.4 (92.5–98.3)	554	90.5 (88.2–92.8)	
AE	4	2.0 (0.7–5.2)	42	6.9 (5.2–9.3)	
C	1	0.5 (0–2.8)	7	1.1 (0.5–2.4)	
G	1	0.5 (0–2.8)	4	0.7 (0.2–1.7)	
AG	1	0.5 (0–2.8)	3	0.5 (0.1–1.4)	
A	0	0 (0–1.9)	2	0.3 (0–1.2)	
Unknown	2	1.0 (0.2–3.7)	0	0 (0–0.6)	
Cue for HIV diagnosis					<0.001
Voluntary testing	102	51.8 (44.8–58.8)	193	31.5 (27.9–35.2)	
Provider-initiated testing	94	47.7 (40.7–54.7)	411	67.2 (63.4–70.9)	
Unknown	1	0.5 (0–2.8)	8	1.3 (0.6–2.6)	
Previous testing					<0.001
Yes	116	58.9 (52.0–65.8)	195	31.9 (28.2–35.6)	
No	57	28.9 (22.6–35.3)	262	42.8 (38.9–46.7)	
Unknown	24	12.2 (7.6–16.8)	155	25.3 (21.9–28.8)	

CI, confidence interval; MSM, men who have sex with men; IDU, intravenous drug user.
[a] By t-test or Pearson's Chi-square test.

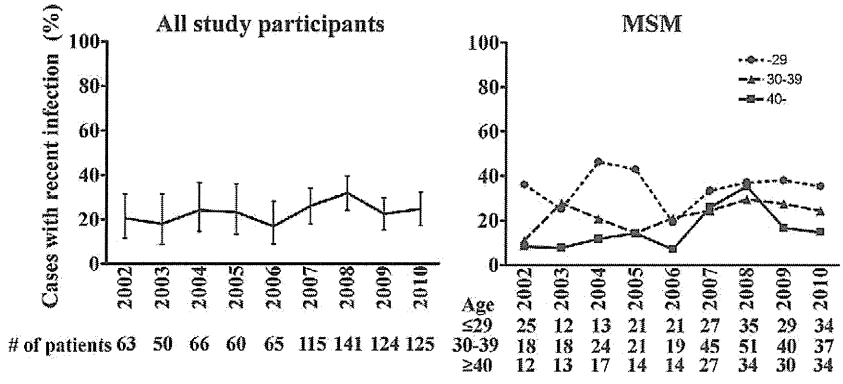

Figure 2. Annual rate of recent infection in newly diagnosed HIV-1-infected cases. The annual rate of recent infection in all study participants (809 patients; left panel), and in men who have sex with men (MSM) categorized by age: ≤29 years (n = 217), 30–39 years (n = 273), and ≥40 years (n = 195) (right panel). The 95% confidence intervals are also shown in the left panel. Data including 95% confidence intervals for the MSM are provided in the **Supplementary Information** (Table S2).

the same strategy in this study and thus defined only BED-positive non-AIDS cases with CD4 cell counts ≥200/μl as recent infection.

In the 456 non-AIDS cases with CD4 cell counts ≥200/μl, 197 cases were BED-positive and classified as recent infection (43.2%; 24.4% of the total cases) (Table 3). BED-negative cases, AIDS cases, and cases with CD4 cell counts <200/μl were classified as chronic infection. Patients with recent infection were younger (33.0 years, 95% CI 31.7–34.3 vs. 37.2 years, 95% CI 36.3–38.1) and had higher CD4 counts (423.2/μl, 95% CI 399.2–447.3 vs. 207.9/μl, 95% CI 193.3–222.4), as expected, and lower viral load (4.61 log VL/ml, 95% CI 4.46–4.76 vs. 4.81 log VL/ml, 95% CI 4.74–4.87), compared to patients with chronic infection. A larger proportion of recent infection (95.4%, 95% CI 92.5–98.3) was caused by HIV-1 subtype B than in those with chronic infection (90.5%, 95% CI 88.2–92.8). There were no significant differences in the nationality and transmission category between recent and chronic infection cases (Pearson's Chi-square test), although the proportion of Japanese patients was higher in recent infection (95.9%, 95% CI 93.2–98.7) than in chronic infection (90.2%, 95% CI 87.8–92.6) (p = 0.012, Chi-square test). A significantly larger proportion of patients underwent previous HIV testing (58.9%, 95% CI 52.0–65.8 vs. 31.9%, 95% CI 28.2–35.6) and were diagnosed with HIV-1 infection by voluntary testing (51.8%, 95% CI 44.8–58.8 vs. 31.5%, 95% CI 27.9–35.2) among recent infection cases than chronic infection cases (p < 0.001 in both, Pearson's Chi-square test).

To delineate the trends in early diagnosis of HIV-1 infection, the annual rate of recent infection in all 809 study participants was plotted over the study period (Figure 2). The rate was stable at ~20% between 2002 and 2010, except for 2007 (26.1%) and 2008 (31.9%), when a slight increase was evident. In order to identify the population that influenced the annual trends of early diagnosis, we focused on MSM patients and again divided them into three age groups: ≤29 years, 30–39 years, and ≥40 years. The rates of recent infection in the ≤29 and ≥40 years MSM groups were the highest and the lowest, respectively, in most years of the study period. The rate in the ≤29 years MSM group was high, ranging from 25.0% to 46.2% between 2002 and 2005, but it decreased to 19.0% in 2006, and increased again in 2007 and remained around 35% between 2007 and 2010. The rate of recent infection in the ≥40-year-old MSM group was steadily low at ~10% between 2002 and 2006, but increased in 2007 to 25.9% and 2008 to 35.3%, then decreased to around 15% in 2009 and 2010. The rate in the 30–39-year-old MSM ranged between those of the other two groups during most part of the study period. These results suggest that younger MSM tend to be diagnosed persistently earlier, whereas older MSM are usually diagnosed at a later stage of the HIV disease.

4. Discussion

The present study analyzed the trends in the proportion of AIDS patients and patients with recent infection among 809 new cases of HIV-1-infection diagnosed between 2002 and 2010. This group recruited from our AIDS Clinical Center represents 20.0% of the total number of newly diagnosed Tokyoites during the same period. We found that MSM, especially younger MSM, tend to be diagnosed at an earlier stage before the development of AIDS, probably because of frequent voluntary HIV testing. The proportion of AIDS cases remained at a steady low level and the rate of recent infection remained at a high level in younger MSM patients, indicating that younger MSM are aware of the risk of their sexual behavior sufficient to take HIV testing repeatedly. On the other hand, in the older MSM, the rate of AIDS was relatively high and the rate of recent infection comparatively low, but transiently increased in 2007 and 2008, suggesting that older MSM with a high-risk of HIV infection usually do not take HIV testing frequently and may respond to campaigns that promote such tests. Interestingly, the Japan Foundation for AIDS Prevention conducted several campaigns to promote voluntary HIV-1 testing in 2007. A popular male Japanese singer took part in one such campaign in July 2007, which was a great surprise among the Japanese in general, and this was followed by an increase in the number of voluntary HIV tests performed in 2007 and 2008.[2] The event may have prompted older MSM at high risk to take voluntary HIV testing, resulting in the transient increase in the rate of early diagnosis for 2007 and 2008. The sharp decline in the rate of early diagnosis observed in 2009 and 2010 in the older MSM group coincided with reductions in the number of voluntary tests,[2] and could be an omen of future increases in the number of AIDS patients in this population. Early diagnosis followed by early introduction of ART may reduce the spread of HIV-1 among MSM, which could help to prevent an HIV epidemic in this population.[6,7,22] A strategy based on the promotion of voluntary testing needs to be formulated, similar to the 2007 campaigns that resulted in significant increases in the rate of early diagnosis in older MSM.

Discordant shifts were observed between the rates of AIDS and recent infection. The reasons may be that AIDS usually develops several years after HIV infection and that disease progression varies enormously among infected individuals. Therefore, the variable length of time during which HIV infection was ignored resulted in the development of AIDS, the proportion of which does not always correlate with the rate of recent infection in the same year.[11] Furthermore, disease progression has been suggested to have become faster in a significant portion of Japanese patients, probably because the prevailing HIV-1 strains in Japan have

adapted to the Japanese population by acquiring escape mutations from immune pressure restricted by human leukocyte antigens (HLAs) popular among the Japanese.[23,24] Based on this point of view, early diagnosis is even more important due to the shorter asymptomatic period before the development of AIDS.

The majority of our study participants were infected with HIV-1 subtype B, and HIV-1 subtype B infection correlated significantly with MSM (crude odds ratio 37.9, $p < 0.001$; Chi-square test). The non-AIDS patients were more likely to be infected with subtype B than AIDS patients (crude odds ratio 1.59, $p = 0.098$). The same was true for recent infection than chronic infection (crude odds ratio 2.81, $p = 0.009$). A previous Japan-wide survey also showed a close relationship between subtype B and MSM in Japan; all cases diagnosed with primary HIV-1 infection ($n = 45$) were caused by subtype B, and such primary infections were significantly frequent among MSM.[14] Considered together, the results indicate that subtype B is the major currently prevalent strain in Japan, especially among MSM, and such strains are probably adapting to the Japanese population by repeated exposure to immune pressure of the Japanese.

This study used case reporting-based surveillance to estimate the number of new HIV-1 infections in Tokyoites between 2002 and 2010. The data were collected at a single center and thus may have included some institutional bias. The study participants were statistically younger and were more likely to be MSM than those of the Tokyo registry. The BED assay was used in this study to determine the rate of recent infection in the selection study group and not to determine the national incidence rate. However, the data from this study suggest the following target-specific differential strategies for controlling the HIV epidemic and for AIDS prevention in Tokyo: campaigns aimed at promoting testing should be directed at older MSM for early diagnosis to prevent/halt the progression of AIDS; commencement of ART for HIV-infected younger MSM at early stages of the disease may effectively reduce the number of new cases based on the control of current hot-spots of HIV transmission among this group.

Acknowledgements

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Conflict of interest: The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2011.11.003.

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AIDS 2012, **26**:397–402

Reply to 'pharmacokinetic concerns related to darunavir/ritonavir plus raltegravir combination therapy trial'

Gervasoni and Cattaneo [1] point out that a potential pharmacokinetic drug–drug interaction between raltegravir and darunavir ultimately affecting the results of the AIDS Clinical Trials Group (ACTG) A5262 trial cannot be ruled out. We agree with this statement, as A5262 was never designed to be a drug–drug interaction study. Indeed, we refrained from categorically rejecting the possibility of significant interactions and simply presented our finding that trough concentrations observed in A5262 were within the range reported in an intensive pharmacokinetic study of darunavir 800/100 mg daily [2].

In A5262, the darunavir troughs were different in the nonvirologic failure (1649 ng/ml) and virologic failure groups (1042 ng/ml), and undetectable raltegravir trough concentrations were associated with increased virologic failure. Importantly, A5262 was not designed to elicit optimal drug exposure variables, nor was it our intention to use the results as a basis for therapeutic drug monitoring (TDM). In applying TDM, the therapeutic range of the drug must first be defined, and this has not been adequately done for raltegravir or darunavir. Along this line, the suggestion by Gervasoni and Cattaneo [1] that darunavir area under the curve (24 h) is the relevant pharmacokinetic parameter raises important questions. What is the optimum exposure of darunavir in HIV-infected patients? Are troughs more important or is the area under the curve? Is there consensus on this exposure variable, and were those data generated from well designed dose-ranging studies or retrospective pharmacokinetic cohort analyses? Clearly, these issues are yet to be resolved in the literature. Without knowing what the ideal darunavir exposure target is, neither our team nor Gervasoni and Cattaneo [1] can state whether virologic failure occurred due to low darunavir troughs, regardless of whether raltegravir caused low darunavir exposure or not. There are in-vitro data to suggest the darunavir protein-binding-corrected 95% inhibitory concentration might be as low as 25 ng/ml [3], which is well below the darunavir trough concentrations in both virologic failure and nonvirologic failure participants in A5262. Previous pharmacokinetic studies of once-daily darunavir found no relevant relationships between darunavir pharmacokinetics and virologic efficacy or safety [4,5].

Gervasoni and Cattaneo [1] also state that no association between raltegravir pharmacokinetics and clinical outcome can be reasonably expected. They apparently based this assertion on raltegravir's pharmacokinetic variability and long residence time on the pre-integration complex, which exceeds the half-life of the complex itself. This presumably makes raltegravir inhibition of the enzyme complex irreversible, which was extrapolated to explain why raltegravir troughs are not related to response. According to Gervasoni and Cattaneo [1], it is unlikely that raltegravir trough concentrations can *per se* directly affect response to therapy of patients enrolled in the ACTG trial. Therefore, they posited that other ways in which raltegravir could indirectly impact on patient outcome, such as an interaction with darunavir, should be advocated. Although there may be a drug–drug interaction, it should be emphatically reiterated that A5262 was not designed to assess this. More importantly, the phase III study of the safety and efficacy of once daily versus twice daily raltegravir in combination therapy for treatment-naïve HIV-infected patients (QDMRK) clearly showed that raltegravir 800 mg once daily was inferior to 400 mg twice daily at 48 weeks [6]; the geometric mean trough concentration$_h$ was 83 nmol/l for once-daily vs. 380 nmol/l for twice-daily dosing. The QDMRK study was a large dose fractionation study, and as such we now know that the raltegravir trough concentrations are indeed an important determinant of therapeutic response. The half-life of drug binding to the integration complex may be an important variable for certain dosing intervals but, if the dosing interval exceeds some threshold, then the integration complex can form and integrate during the period when raltegravir concentrations are low or absent. This is further demonstrated by data with dolutegravir (same mechanism of action as raltegravir), which exhibited a concentration–response relationship when a wide range of doses (25-fold) was used in early development [7]. A recent article demonstrates the dissociative half-life of dolutegravir is 71 h, considerably longer than the 8.8 h for raltegravir [8]. Thus, a concentration–response relationship was still determined for dolutegravir, even though the enzyme complex off-rate was eight times slower. Gervasoni and Cattaneo [1] are incorrect in assuming that the raltegravir trough concentrations do not affect response to therapy in A5262, and we cannot confirm nor rule out a drug–drug interaction between darunavir and raltegravir in this particular study.

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Conflicts of interest

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