



**HAL**  
open science

## **KSHV DNA viraemia correlates with low CD4+ cell counts in Italian males at the time of diagnosis of HIV infection**

Saverio Giuseppe Parisi, Caterina Boldrin, Samantha Andreis, Roberto Ferretto, Rodolfo Fuser, Marina Malena, Vinicio Manfrin, Sandro Panese, Renzo Scaggiante, Luca Dori, et al.

► **To cite this version:**

Saverio Giuseppe Parisi, Caterina Boldrin, Samantha Andreis, Roberto Ferretto, Rodolfo Fuser, et al.. KSHV DNA viraemia correlates with low CD4+ cell counts in Italian males at the time of diagnosis of HIV infection. *Journal of Medical Virology*, 2011, 83 (3), pp.384. 10.1002/jmv.21987 . hal-00613772

**HAL Id: hal-00613772**

**<https://hal.science/hal-00613772>**

Submitted on 6 Aug 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



## KSHV DNA viraemia correlates with low CD4+ cell counts in Italian males at the time of diagnosis of HIV infection

Journal:	<i>Journal of Medical Virology</i>
Manuscript ID:	JMV-10-1763.R2
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	27-Sep-2010
Complete List of Authors:	<p>Parisi, Saverio; Padova University, Department of Histology, Microbiology and Medical Biotechnology            Boldrin, Caterina; Padova University, Department of Histology, Microbiology and Medical Biotechnology            Andreis, Samantha; Padova University, Department of Histology, Microbiology and Medical Biotechnology            Ferretto, Roberto; Schio Hospital, Infectious Diseases Unit            Fuser, Rodolfo; Treviso Hospital, Infectious Diseases Unit            Malena, Marina; ULSS 20 Verona, Center of Preventive Medicine            Manfrin, Vinicio; Vicenza Hospital, Infectious Diseases Unit            Panese, Sandro; Venezia Hospital, Infectious Diseases Unit            Scaggiante, Renzo; Padova Hospital, Infectious Diseases Unit            Dori, Luca; Clinica Infectious Disease, Dept. Public Health, Tor Vergata University            Sarmati, Loredana; Clinica Infectious Disease, Dept. Public Health, Tor Vergata University            Biasolo, Maria Angela; Padova University, Department of Histology, Microbiology and Medical Biotechnology            Nicastrì, Emanuele; Istituto Nazionale di Malattie Infettive Lazzaro Spallanzani, Infectious Diseases Unit            Andreoni, Massimo; Tor Vergata University, Infectious Diseases Department            Cruciani, Mario; ULSS 20 Verona, Center of Preventive Medicine            Palù, Giorgio; University of Padova, Department of Histology, Microbiology and Medical Biotechnologies</p>
Keywords:	KSHV-DNA, HIV, immunodeficiency, homosexual relationship

SCHOLARONE™  
Manuscripts

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Peer Review

**Table 1.** Demographic, virological, and immunological characteristics of untreated HIV-seropositive patients according to the presence of KSHV DNA in PBMCs.

	All patients (n=481)	KSHV neg (n=407)	KSHV pos (n=74)	p value
Mean age in years ( $\pm$ SD)	40.3 ( $\pm$ 11.2)	41.7 ( $\pm$ 11.7)	40.0 ( $\pm$ 11.1)	0.26
Risk factor for HIV infection:				
*unprotected homosexual relationship, (%)	282 (64.4)	226 (61.4)	56 (80.0)	0.003
Patients with primary HIV infection, n. (%)	102 (21.2)	92 (22.6)	10 (13.7)	0.09
**Patients with B subtype (%)	401 (88.5)	339 (88.1)	62 (91.2)	0.45
Mean CD4+ cell count / $\mu$ l ( $\pm$ SD)	449 ( $\pm$ 288)	461 ( $\pm$ 291)	389 ( $\pm$ 264)	0.06
Patient CD4+ cell count, n (%)				0.08
<200 cells	87 (18.1)	70 (17.2)	18 (24.3)	
200-350 cells	112 (23.3)	89 (21.9)	22 (29.7)	
>350 cells	282 (58.6)	248 (60.9)	34 (46.0)	0.025
Percentage of CD4+ cells ( $\pm$ SD)	22.1 ( $\pm$ 10.6)	22.3 ( $\pm$ 10.5)	21.2 ( $\pm$ 10.6)	0.44
HIV RNA cp/ml ( $\pm$ SD)	268061 ( $\pm$ 872183)	280623 ( $\pm$ 940158)	200150 ( $\pm$ 304285)	0.50
HIV DNA cp x 10 <sup>6</sup> PBMCs ( $\pm$ SD)	2893 ( $\pm$ 4429)	2799 ( $\pm$ 4483)	3340 ( $\pm$ 4162)	0.38

\* % of 438 evaluable patients

\*\* % of 453 evaluable data

1 **Table 2**

2 Univariate analysis of 60 untreated HIV-seropositive patients with KSHV DNA  
 3 in PBMCs and for whom plasma samples were available/analysed  
 4

	All patients (n=60)	KSHV pl pos (n=33)	KSHV pl neg (n=27)	<i>P</i> value
Mean age in years ( $\pm$ SD)	42.1 ( $\pm$ 11.5)	44.1 ( $\pm$ 12.8)	39.2 ( $\pm$ 8.8)	0.10
Risk factor for HIV infection: unprotected homosexual relationship (%)	47 (78)	29 (87.9)	18(66.6)	0.37
Patients with primary HIV infection, n. (%)	7 (11.6)	5 (15.1)	2 (7.4)	0.46
Patients with B subtype (%)	55 (91.6)	29 (87.8)	26 (96.3)	0.27
CD4+ cell count/ $\mu$ l ( $\pm$ SD)	404 ( $\pm$ 267)	325 ( $\pm$ 258)	513 ( $\pm$ 244)	0.006
Patient CD4+ cell count, n. (%)				0.002
<200 cells	13 (21.7)	13 (39.3)	0 (0)	
200-350 cells	19 (31.7)	9 (27.2)	10 (37.0)	
>350 cells	28 (46.6)	11 (33.3)	17 (63.0)	0.01
Percentage of CD4+ cells ( $\pm$ SD)	22.1 ( $\pm$ 10.4)	19.2 ( $\pm$ 11.4)	25.8 ( $\pm$ 7.7)	0.019
HIV RNA cp/ml ( $\pm$ SD)	197691 ( $\pm$ 311073)	224658 ( $\pm$ 313325)	162095 ( $\pm$ 310812)	0.45
HIV DNA cp x 10 <sup>6</sup> PBMCs ( $\pm$ SD)	3446 ( $\pm$ 4362)	3252 ( $\pm$ 3786)	3786 ( $\pm$ 5407)	0.67

5

1 **Table 3** Multivariate analysis of untreated HIV-seropositive patients with KSHV DNA in  
2 PBMCs according to the presence of KSHV DNA in plasma  
3  
4

	p	OR	95% CI
Patients with age > median age	0.70	0.06	-0.24-0.35
Risk factor for HIV infection unprotected homosexual relationship	0.55	0.09	-0.22-0.40
CD4+ cell count/ml	0.03	0.001	<0.001-0.001
Patients with >median HIV RNA cp	0.28	-0.17	-0.48-0.14

1  
2  
3 1 **KSHV DNA viraemia correlates with low CD4+ cell count in Italian males**  
4  
5  
6 2 **at the time of diagnosis of HIV infection**  
7  
8  
9 3

10  
11 4 Saverio G. Parisi<sup>a\*</sup>, Caterina Boldrin<sup>a</sup>, Samantha Andreis<sup>a</sup>, Roberto Ferretto<sup>b</sup>, Rodolfo Fuser<sup>c</sup>,  
12 5 Marina Malena<sup>d</sup>, Vinicio Manfrin<sup>e</sup>, Sandro Panese<sup>f</sup>, Renzo Scaggiante<sup>g</sup>, Luca Dori<sup>h</sup>, Loredana  
13 6 Sarmati<sup>h</sup>, Maria A. Biasolo<sup>a</sup>, Emanuele Nicastrì<sup>i</sup>, Massimo Andreoni<sup>h</sup>, Mario Cruciani<sup>d</sup>,  
14 7 Giorgio Palù<sup>a</sup>  
15  
16  
17  
18  
19  
20  
21 8

22  
23 9 <sup>a</sup>Department of Histology, Microbiology and Medical Biotechnology, Padua University, Via  
24 10 Gabelli 63, 35100 Padova, Italy

25  
26  
27  
28 11 <sup>b</sup>Infectious Disease Unit, City Hospital, via De Lellis, 36015 Schio

29  
30 12 <sup>c</sup>Infectious Disease Unit, City Hospital, piazza Ospedale 1, 31100 Treviso

31  
32 13 <sup>d</sup>Center of Preventive Medicine, ULSS 20, via Germania 20, 37135 Verona,

33  
34 14 <sup>e</sup>Infectious Disease Unit, San Bortolo Hospital, via Rodolfi 37, 36100 Vicenza

35  
36 15 <sup>f</sup>Infectious Disease Unit, City Hospital, via Paccagnella 11, 30170 Mestre-Venezia

37  
38 16 <sup>g</sup>Infectious Disease Unit, Padova Hospital, via Ospedale 1, 35100 Padova

39  
40 17 <sup>h</sup>Infectious Disease Unit, Tor Vergata University, V. Montpellier 1, 00133, Roma.

41  
42 18 <sup>i</sup>Istituto Nazionale di Malattie Infettive Lazzaro Spallanzani, via Portuense 292, 00149 Roma  
43  
44  
45  
46  
47  
48  
49  
50

51 20 \*Corresponding Author: Parisi Saverio Giuseppe, MD.

52 21 Department of Histology, Microbiology and Medical Biotechnology, Via Gabelli 63, 35100

53  
54 22 Padova, Italy. tel +390498272344, fax +390498272355, e-mail: [saverio.parisi@unipd.it](mailto:saverio.parisi@unipd.it)  
55

56 23 **Short title:** KSHV viraemia in patients infected with HIV  
57  
58  
59 24  
60  
25

## Abstract

To evaluate the relevance, virological and immunological markers of Kaposi sarcoma herpesvirus 8 (KSHV) viraemia in Italian male patients at the time of diagnosis of infection with HIV-1, 481 men infected with HIV were recruited consecutively. The presence of KSHV DNA was evaluated in peripheral blood mononuclear cells (PBMCs) and in plasma and correlated with demographic and viro-immunological parameters.

Seventy-four patients had KSHV DNA detected in PBMCs. By univariate analysis, the presence of KSHV DNA was associated significantly with unprotected homosexual relationships ( $p=0.003$ ) and it was significantly higher in patients with  $CD4^+$  cell  $<350$  ( $p=0.025$ ). By multivariate analysis, homosexual relationships were associated independently with KSHVDNA in PBMCs (OR: 3.25; 95% CI: 1.1-9.7;  $p=0.035$ ). Among the 74 patients with KSHVDNA detected in PBMCs, plasma samples from 60 were analysed and 33 were positive for KSHV DNA. The  $CD4^+$  cell counts and percentages were significantly lower in patients with KSHV DNA in both PBMCs and plasma as compared to patients with only KSHV DNA in PBMCs ( $p=0.006$  and  $p=0.019$ , respectively). Among the patients with KSHV DNA detected in PBMCs, all 13 patients with  $CD4^+$  cells count  $<200$  had detectable levels of KSHV in their plasma. By multivariate analysis adjusted for the epidemiologic and virological parameters, low  $CD4^+$  cell count was the only independent variable associated with the presence of KSHV DNA in plasma (OR, 0.001; 95% CI,  $<0.001-0.001$ ;  $p=0.03$ ).

In HIV-positive antiretroviral therapy-naïve males, KSHV active replication as detected by KSHV DNA in plasma was associated significantly with low  $CD4^+$  cell count.

Key words: KSHV DNA, HIV, immunodeficiency, homosexual relationship.

## Introduction

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 Kaposi's Sarcoma-associated herpesvirus (KSHV) is linked causally to primary effusion  
4 lymphoma, multicentric Castleman disease, and all epidemiological forms of Kaposi's  
5 sarcoma (KS) (Boshoff and Weiss, 1998). The prevalence of KSHV varies between countries  
6 and populations. Variable routes of transmission, both sexual and non-sexual, have been  
7 described (Schulz & Moore, 1999; Hengge et al., 2002). In areas with low seroprevalence,  
8 KSHV appears to be acquired predominantly through sexual contact, and sex between men  
9 may be an important route of transmission (Schulz & Moore, 1999). Familial clustering of  
10 KSHV is observed frequently in endemic regions, where nasal secretions and saliva may be  
11 the sources of KSHV transmission (Plancoulaine et al., 2002; Andreoni et al., 1999; Rezza et  
12 al., 2000).

13 Variable seroprevalence of KSHV has been reported among subjects infected with HIV  
14 (Campbell et al., 1999; Laney et al., 2004)). In Italy, its overall prevalence in HIV-  
15 seropositive people in 1997-98 was demonstrated to be 31.4%, an increase from the 1987-88  
16 prevalence of 14.6%, suggesting a spread of infection among HIV-seropositive subjects, and  
17 possibly from them to the HIV-negative community, by sexual and non-sexual routes (Parisi  
18 SG, Sarmati L et al., 2002).

19 HIV and KSHV are able to influence each other, and there is in vitro evidence that suggest  
20 the ability of one virus to facilitate the replication of the other. HIV activates lytic cycle  
21 replication of KSHV, and KSHV lytic antigens interact with HIV *Tat* to increase the cell's  
22 susceptibility to HIV infection (Zeng et al., 2007; Varthakavi et al., 2002). In vivo, HIV-1  
23 infection has been associated with the presence of KSHV in any mucosal surface, and the  
24 presence of KSHV DNA in PBMCs is predictive of the development of KS in patients  
25 infected with HIV (Moore et al., 1996; Taylor et al., 2004, Engels et al., 2003). KSHV-DNA  
26 was more detected frequently in people infected with HIV and in the oropharynx and

1 peripheral blood of Ugandans with endemic and epidemic KS (Johnston et al., 2009). KSHV  
2 DNA is detectable in KS tissues (Chang et al., 1994), in PBMCs from patients with KS and  
3 multicentric Castleman disease, and in PBMCs and effusion fluid samples from patients with  
4 primary effusion lymphoma (Marcelin et al., 2007). Among KS patients, KSHV DNA has  
5 been detected in both plasma and PBMCs, and often no consistent difference between  
6 biological compartments is detected (Lin et al 2009). KSHV-infected cells were detected at a  
7 very low frequency in the inguinal lymph nodes of HIV-1-seropositive subjects without  
8 KSHV-associated diseases (Campbell et al., 2005), suggesting that the latent infection of  
9 lymph node cells provides a mechanism for the persistence of KSHV in KSHV/HIV-1-co-  
10 infected people.

11 Although effective HIV suppression has been correlated with the regression of KS after  
12 antiretroviral therapy (Martinez et al., 2006), the persistence of KS despite seemingly  
13 effective antiretroviral therapy (e.g., an undetectable HIV load) is not rare (Krown et al.,  
14 2008). However, the interactions between immune responses, KSHV replication, and their  
15 relative contribution to KS have not been well characterised to date.

16 The aim of the study was to evaluate the prevalence of KSHV DNA viraemia in male patients  
17 from northeastern Italy with chronic and acute HIV-1 infections at the time of HIV diagnosis.  
18 Moreover, correlations between KSHV viraemia and the virological and immunological  
19 parameters of HIV infection were evaluated.

20

## Materials and Methods

### Study population

Four hundred and eighty one men infected with HIV-1 were enrolled at six Infectious Diseases Units located in Veneto, Italy. The patients were recruited consecutively from July 1, 2004 to December 31, 2008 after obtaining their written informed consent to participate in the study. The ethics committee of the hospitals gave approval for the study.

The eligibility criteria included being of Italian origin, >18 years of age, and having antiretroviral-drug-naïve status.

Blood samples were submitted to the Laboratory of Virology at the University of Padua, stored within 6 hours from collection, and analysed subsequently.

A primary or recent HIV infection was defined by the presence of either of the following: (i) a negative or indeterminate HIV antibody-ELISA associated with HIV RNA-positive plasma; or (ii) an initially negative test for HIV antibodies followed by positive serology within 18 months.

The HIV RNA plasma viral load assessment (using Roche Cobas AmpliPrep-Cobas TaqMan HIV-1, version 1 assay, F. Hoffmann-La Roche, Diagnostics Division, Basel, Switzerland) and HIV genotypic analysis to detect subtype were performed as described previously (Parisi et al., 2007).

CD4+ cell counts and percentages were determined at enrolment. Blood collected in EDTA was separated into plasma and cells by Ficoll-Paque Plus density gradient centrifugation.

Aliquots of plasma and dry pellets of  $2 \times 10^6$  PBMCs were stored at  $-80^\circ\text{C}$  until use.

### KSHV DNA analysis

The extraction and purification of DNA from cells and plasma samples was performed using the QIAmp Blood kit (Qiagen, Inc., Chatsworth, CA). KSHV DNA from cell and plasma samples was detected using a real-time polymerase chain reaction (RT-PCR; with TaqMan

1 probe, Applied Biosystems, Foster City, CA) of open reading frame 26 (ORF26), as described  
2 previously (White and Campbell, 2000). The nucleotide sequence targeted by the primers and  
3 probes is conserved highly among the three major subgroups of KSHV (Poole et al., 1999).  
4 During all DNA extractions and purifications, precautions were taken to reduce the risk of  
5 false-positive results.

#### 6 Cellular HIV DNA quantitation

7 The Real-Time TaqMan protocol published by Viard and colleagues (Viard et al., 2004) was  
8 used to quantify the HIV DNA copy number in PBMCs. The cell line 8E5, containing one  
9 copy of integrated HIV DNA in each cell, was used to build a standard curve. The sensitivity  
10 of the test was 13.3 copies/10<sup>6</sup> PBMCs.

#### 11 Statistical analysis

12 The variables initially taken into account were age, risk factors for HIV infection (men who  
13 had sex with a man vs. other risk groups), primary HIV infection, HIV subtype (B subtype vs.  
14 other subtypes), CD4+ cell count, HIV RNA, and HIV DNA load. Among these, age was a  
15 quantitative variable, while primary HIV infection, HIV subtype, and the presence of drug  
16 resistance mutations were binary (yes/no) variables and were coded as 1/0. CD4+ cell count,  
17 HIV RNA, and HIV DNA were both quantitative and discrete variables.

18 Univariate analyses were performed using all of the covariates, taking them both (when  
19 appropriate) as continuous variables, as well as categorising by clinically relevant values. Chi  
20 square and Kruskal-Wallis tests were used to assess differences between patient groups with  
21 reference to categorical and continuous variables, respectively. The significance level was set  
22 at 0.05 and all *P* values were two-tailed. To obtain an adjusted analysis while accounting for  
23 all possible risk factors, a multiple logistic regression model was used. Age, HIV-RNA level,  
24 and all of the significant variables in the univariate analysis were included in the multivariable  
25 model.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1 All of the analyses were performed on an intention-to-treat basis using SPSS for Windows  
2 13.0 (SPSS Inc, Chicago, Illinois).

For Peer Review

26

## Results

Of the 481 patients enrolled, in 43 cases (8.9%) it was not possible to define their risk for HIV infection. Of the remaining 438 patients, 282 were homosexual, 90 were heterosexual and 66 were intravenous drug abusers. Additionally, 102 patients had a primary HIV infection and 379 patients had a chronic infection.

Patients with chronic HIV infections were older ( $41 \pm 11.4$  vs.  $37.6 \pm 10.2$  years,  $p=0.008$ ) and had CD4+ cell counts ( $413.8 \pm 273.4$  vs.  $603.9 \pm 297.5$  cells/ $\mu$ l,  $p<0.001$ ) and percentages ( $21.2 \pm 10.2$  vs.  $26.6 \pm 10.5\%$ ,  $p<0.001$ ) significantly lower than the patients with primary infections.

Table 1 presents the population of patients infected with HIV studied according to the presence of KSHV DNA in PBMCs and different epidemiological and viro-immunological parameters.

Of the 481 enrolled patients, 74 (15.4%) had KSHV DNA detected in their PBMCs. By univariate analysis, unprotected homosexual relationships and a CD4+ cell count  $<350$  cells/ $\mu$ l were associated significantly with the presence of KSHV DNA in PBMCs ( $p=0.003$  and  $p=0.025$ , respectively).

Patients with primary HIV infections had a lower (but not significantly lower) rate of KSHV DNA positivity in PBMCs in comparison to patients with chronic infections. No significant differences in HIV RNA or HIV DNA levels were found between KSHV DNA-positive and KSHV DNA-negative patients.

By multivariate analysis adjusted for different epidemiological and virological parameters, having a homosexual relationship (OR: 3.25; 95% CI: 1.1-9.7;  $p=0.035$ ) was the only parameter that was associated independently with the presence of KSHV DNA in PBMCs.

1 Table 2 presents the univariate analysis of the population of HIV/KSHV co-infected male  
2 patients studied in relation to the simultaneous presence of KSHV DNA in PBMCs and  
3 plasma.

4 Among the 74 subjects with KSHV DNA detected in PBMCs, the presence of KSHV DNA in  
5 plasma was analysed for 65 subjects from whom plasma specimens were available. Of the 65  
6 patients, 38 (58.4%) also had KSHV DNA-positive plasma. Overall, five patients had an  
7 ultimate diagnosis of KS and were excluded from the evaluation. Among the 60 non-KS  
8 patients, 33 (55%) had KSHV DNA detected in both PBMCs and plasma; 28 (85%) of these  
9 patients had a chronic HIV infection and 5 had a primary HIV infection. The CD4+ cell  
10 counts and percentages were significantly lower in patients with detectable levels of KSHV  
11 DNA in both PBMCs and plasma compared to patients with KSHV DNA detectable only in  
12 PBMCs ( $p=0.006$  and  $p=0.019$ , respectively). Moreover, among patients with KSHV DNA  
13 detected in PBMCs, all 13 subjects with CD4+ cell counts  $<200$  cells/ $\mu$ l also had KSHV DNA  
14 detectable in plasma.

15 In the multivariate analysis (Table 3) adjusted for the different epidemiologic and virological  
16 parameters, CD4+ cell count was the only independent variable associated with the presence  
17 of KSHV DNA in the plasma of co-infected HIV/KSHV patients (OR, 0.001; 95% CI,  
18  $<0.001-0.001$ ;  $p=0.03$ ).

19 A subgroup of 20 patients with KSHV DNA detected in PBMCs and plasma were re-analysed  
20 for the presence of KSHV DNA in PBMCs and plasma in a follow-up study.

21 Nine subjects still had KSHV DNA detectable in PBMCs and plasma after a mean time of 19  
22 months. Six of these subjects were still highly active antiretroviral therapy (HAART)-naïve  
23 and three were on HAART, two of whom were HIV plasma viraemic at the time of the second  
24 analysis. Of the 14 patients who were successfully HAART-treated ( $<50$  HIV RNA  
25 copies/ml), 11 were KSHV DNA-negative in PBMCs and plasma after a mean time of 17  
26 months.

1  
2  
3 1 Moreover, among the 14 patients treated with HAART, the 11 patients who were negative for  
4  
5 2 KSHV DNA had a mean increase in their CD4+ cell counts of 249 cells/ $\mu$ l (range 57-603),  
6  
7  
8 3 whereas the 3 treated patients with detectable levels of KSHV DNA had CD4+ cell count  
9  
10 4 increases of 57, 97, and 74 cells/ $\mu$ l.  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Peer Review

## Discussion

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

The presence of KSHV DNA in a cohort of patients at the time of their initial diagnosis of HIV infection was studied. Out of the 481 evaluated subjects, 74 (15.3%) were found to have detectable levels of KSHV DNA in their PBMCs. Approximately half of the subjects found to be PBMC-positive had concomitant active KSHV replication in their plasma.

The presence of KSHV DNA in PBMCs is predictive of the development of KS in HIV-infected patients, and KSHV DNA has been detected in up to 70% of HIV-related KS cases (Whitby et al., 1995; Moore et al., 1996). Previous studies have reported variable results on the prevalence of KSHV DNA in HIV-1-infected people without KS. Laney and colleagues found KSHV DNA in the PBMCs of 28.9% of patients without KS, a rate almost double that of the cohort in this study (Laney et al., 2004). By contrast, Campbell et al (1999) found that 18% of KSHV/HIV-1 co-infected people without KS have detectable KSHV DNA in their PBMCs. The lower prevalence in the cohort of this study may be due to the distribution of risk factors for HIV, with only 67% of our subjects being homosexual compared to >90% in the previous studies. The median CD4+ cell values were lower in the studies by Campbell and by Laney than they were in the cohort of this study.

In this study, patients with detectable KSHV DNA had lower CD4+ cells count compared to patients without detectable KSHV DNA. Using multivariate analysis, CD4+ cell count was found to be correlated negatively with active KSHV replication. By contrast, any correlation between KSHV DNA detection and either HIV RNA, HIV DNA viral load, or HIV subtype was not found. The lack of a correlation between the presence of KSHV DNA and HIV viraemia seems to confirm previous observations that assign a greater role to the immune response (in particular the NK-type response) and CD4+ cell recovery rather than to HIV replication in the control of KS in AIDS patients (Sirianni et al., 2002; Cattelan et al., 2001). Higher levels of KSHV DNA (although not significantly so) were found in the group of

1 subjects with chronic HIV infections than in those with acute infections. This result agrees  
2 with the significant correlation between KSHV DNA and low CD4+ cell count and seems to  
3 confirm that it is the immunological deficiency (which is more pronounced in advanced HIV  
4 infection) rather than the level of HIV replication that favours KSHV viraemia. The relevance  
5 of the immune response to the control of KSHV replication is also demonstrated by the results  
6 of a study in which KS risk was found to be associated positively with a reduced number of  
7 lymphocytes, including CD4-positive cells (<457 cells/mL;) (Brown et al., 2006).

8 Almost 60% of patients with KSHV DNA-positive PBMCs also showed the presence of  
9 HHV8 in plasma. Absolute CD4+ cell counts and percentages were significantly lower in  
10 patients who were KSHV DNA-positive in both plasma and PBMCs compared to patients  
11 positive only in PBMCs. Again, the active replication of KSHV was associated significantly  
12 with a low number and low percentage of CD4+ cells, and this confirms the more relevant  
13 role of immune response in controlling KSHV replication.

14 Previous studies have indicated a correlation between KSHV replication and KS pathogenesis  
15 (Whitby et al., 1995; Cannon et al., 2003; Lorenzen et al., 2002; Smith et al., 1997; Broccolo  
16 et al., 2002; Engels et al., 2003). In individuals infected with HIV the use of HAART has a  
17 role in reducing the frequency of KSHV viraemia, mostly because of the immuno-restorative  
18 properties of HIV treatment (Gill et al., 2002; Rizzieri et al., 1997; Lebbe et al., 1998).  
19 Treatment with a regimen that includes a protease inhibitor was associated with the clearance  
20 of KSHV DNA in KS lesions and PBMCs, and with the regression of KS lesions in AIDS  
21 patients (Lebbe et al., 1998; Blum et al., 1997). Furthermore, a number of reports have linked  
22 tumour regression after the initiation of HAART to the restoration of immune function  
23 (Dupont et al., 2000; Marcelin et al., 2004), and it has been reported that KSHV DNA amount  
24 in PBMCs rebounded in patients with KS after a short interruption of efficient antiretroviral  
25 therapy (Parisi et al., 2002).

1  
2  
3 1 Longitudinal observations on KSHV viraemia are limited. In a large cohort of homosexual  
4  
5 2 men who seroconverted to KSHV antigens, KSHV viraemia persisted for up to 6 months  
6  
7  
8 3 (Goudsmit et al., 2000). This issue was addressed in a subgroup of patients from the cohort of  
9  
10 4 this study. KSHV viraemia persisted in the majority of patients who were untreated or treated  
11  
12 5 unsuccessfully with HAART. By contrast, all of the patients on successful HAART cleared  
13  
14 6 KSHV from their blood after a mean period of 17 months.

15  
16  
17 7 Before drawing any conclusions, some potential biases of the study must be addressed. The  
18  
19 8 cross-sectional design of the study does not allow conclusive explanation of the correlation  
20  
21 9 between KSHV viraemia and low CD4+ cell count. However, although the small number of  
22  
23 10 patients enrolled in this longitudinal study may limit its conclusiveness, the low CD4+ cell  
24  
25 11 counts in patients on HAART was associated significantly with active replication of KSHV.  
26  
27 12 Studies in a larger population are required to confirm this result. Finally, the lack of risk  
28  
29 13 factors for HIV infection for less than 10% of the patients may have caused misclassification,  
30  
31 14 potentially biasing the correlation between unprotected homosexual relationships and KSHV  
32  
33 15 viraemia.

34  
35  
36 16 In conclusion, KSHV DNA-positive results were observed in approximately 15% of an HIV-  
37  
38 17 positive cohort of Italian male subjects. The active replication of KSHV was associated  
39  
40 18 significantly with a low number of CD4+ cells, and it was more frequent in patients with  
41  
42 19 chronic than acute HIV infections. A longitudinal study of a limited number of patients  
43  
44 20 demonstrated a direct correlation between immune recovery during HAART and the  
45  
46 21 disappearance of KSHV DNA.  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3

**Potential conflicts of interest: No conflicts**

For Peer Review

**References**

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
- 1  
2 Anderson LA, Lauria C, Romano N, Brown EE, Whitby D, Graubard BI, Li Y, Messina A,  
3 Gafà L, Vitale F, Goedert JJ. 2008. : Risk factors for classical Kaposi sarcoma in a  
4 population-based case- control study in Sicily. *Cancer Epidemiol Biomarkers Prev.*  
5 *Dec;17(12):3435-43.*  
6  
7 Andreoni M, El-Sawaf G, Rezza G, Ensoli B, Nicastrì E, Ventura L, Ercoli L, Sarmati L,  
8 Rocchi G 1999. High seroprevalence of antibodies to human herpesvirus-8 in Egyptian  
9 children: evidence of nonsexual transmission. *J Natl Cancer Inst;91: 465–9.*  
10  
11 Blum L, Pellet C, Agbalika F, Blanchard G, Morel P, Calvo F, Lebbé C. 1997,. Complete  
12 remission of AIDS-related Kaposi's sarcoma associated with undetectable human herpesvirus  
13 8 sequences during anti-HIV protease therapy. *AIDS 11: 1653-1655.*  
14  
15 Boshoff C, Weiss RA. 1998. Kaposi's sarcoma associated herpesvirus. *Adv Cancer Res;*  
16 *75:57-86.*  
17  
18 Broccolo F, Bossolasco S, Careddu AM, Tambussi G, Lazzarin A, Cinque P. 2002. Detection  
19 of DNA of lymphotropic herpesviruses in plasma of human immunodeficiency virus-infected  
20 patients: frequency and clinical significance. *Clin Diagn Lab Immunol; 9:1222-8.*  
21  
22 Brown EE, Whitby D, Vitale F, Marshall V, Mbisa G, Gamache C, Lauria C, Alberg AJ,  
23 Serraino D, Cordiali-Fei P, Messina A, Goedert JJ. 2006. Virologic, hematologic, and  
24 immunologic risk factors for classic Kaposi sarcoma. *Cancer. Nov 1;107(9):2282-90.*  
25

- 1  
2  
3 1 Campbell TB, Fitzpatrick L, MaWhinney S, Zhang XQ, Schooley RT 1999. Human  
4  
5 2 herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus) infection in men receiving treatment  
6  
7 3 for HIV-1 infection. *J Acquir Immune Defic Syndr*; 22:333–40.  
8  
9 4  
10  
11 5 Campbell TB, Staskus KA, Folkvord J, White IE, Neid J, Zhang XQ, Connick E. 2005. .:  
12  
13 6 Persistence of Kaposi sarcoma-associated herpesvirus (KSHV)-infected cells in KSHV/HIV-  
14  
15 7 1-coinfected subjects without KSHV-associated diseases. *J Infect Dis.*191(3):367-71.  
16  
17 8  
18  
19 9 Cannon MJ, Dollard SC, Black JB, Edlin BR, Hannah C, Hogan SE, Patel MM, Jaffe HW,  
20  
21 10 Offermann MK, Spira TJ, Pellett PE, Gunthel CJ. 2003. .: Risk factors for Kaposi's sarcoma  
22  
23 11 in men seropositive for both human herpesvirus 8 and human immunodeficiency virus. *AIDS*;  
24  
25 12 17:215-22.  
26  
27 13  
28  
29 14 Cattelan AM, Calabrò ML, Gasperini P, Aversa SM, Zanchetta M, Meneghetti F, De Rossi A,  
30  
31 15 Chieco-Bianchi L. 2001: Acquired immunodeficiency syndrome-related Kaposi's sarcoma  
32  
33 16 regression after highly active antiretroviral therapy: biologic correlates of clinical outcome. *J*  
34  
35 17 *Natl Cancer Inst Monogr.* 28:44-9  
36  
37 18  
38  
39 19 Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS.  
40  
41 20 1994. Identification of Herpesvirus-like DNA sequences in AIDS-associated Kaposi's  
42  
43 21 sarcoma. *Science* 266 :1865-9  
44  
45 22  
46  
47 23 Dupont C, Vasseur E, Beauchet A, Aegerter P, Berthé H, de Truchis P, Zucman D, Rouveix  
48  
49 24 E, Saiag P. 2000. Long-term efficacy on Kaposi's sarcoma of highly active antiretroviral  
50  
51 25 therapy in a cohort of HIV-positive patients. *AIDS*, 14: 987-993.  
52  
53  
54  
55  
56  
57  
58  
59  
60

26

- 1  
2  
3 1 Engels EA, Biggar RJ, Marshall VA, Walters MA, Gamache CJ, Whitby D, Goedert JJ. 2003.  
4  
5 2 Detection and quantification of Kaposi's sarcoma-associated herpesvirus to predict AIDS-  
6  
7 associated Kaposi's sarcoma. *AIDS*; 17:1847-51.  
8  
9  
10 4  
11  
12 5 Gill J, Bourboulia D, Wilkinson J, Hayes P, Cope A, Marcelin AG, Calvez V, Gotch F,  
13  
14 Boshoff C, Gazzard B. 2002. .: Prospective study of the effects of antiretroviral therapy on  
15  
16 Kaposi Sarcoma-associated Herpesvirus infection in patients with and without Kaposi  
17  
18 Sarcoma. *J Acquir Immune Defic Syndr* 31:384-90.  
19  
20  
21  
22 9  
23  
24 10 Goudsmit J, Renwick N, Dukers NH, Coutinho RA, Heisterkamp S, Bakker M, Schulz TF,  
25  
26 Cornelissen M, Weverling GJ. 2000. .: Human herpesvirus 8 infections in the Amsterdam  
27  
28 Cohort Studies (1984-1997): analysis of seroconversions to ORF65 and ORF73. *Proc Natl*  
29  
30 *Acad Sci U S A*; 97:4838-43.  
31  
32  
33  
34 14  
35  
36 15 Hengge UR, Ruzicka T, Tyring SK, Stuschke M, Roggendorf M, Schwartz RA, Seeber S.  
37  
38 2002. Update on Kaposi's sarcoma and other HHV8 associated diseases. Part 1:  
39  
40 epidemiology, environmental predispositions, clinical manifestations, and therapy. *Lancet*  
41  
42 *Infect Dis*; 2, 281–292  
43  
44  
45  
46 19  
47  
48 20 Johnston C, Orem J, Okuku F, Kalinaki M, Saracino M, Katongole-Mbidde E, Sande M,  
49  
50 Ronald A, McAdam K, Huang ML, Drolette L, Selke S, Wald A, Corey L, Casper C. 2009.  
51  
52 22 Impact of HIV infection and Kaposi sarcoma on human herpesvirus-8 mucosal replication and  
53  
54 dissemination in Uganda. *PLoS One*.;4(1):e4222. Epub 2009 Jan 20.  
55  
56  
57  
58 24  
59  
60 25 Krown SE, Lee JY, Dittmer DP, AIDS Malignancy Consortium 2008. More on HIV-  
26 associated Kaposi's Sarcoma *New Engl J Med*; 358(5):535-6

- 1  
2  
3 1  
4  
5  
6 2 Laney AS, Dollard SC, Jaffe HW, Offermann MK, Spira TJ, Gunthel CJ, Pellett PE, Cannon  
7  
8 3 MJ. 2004. Repeated measures study of human herpesvirus 8 (HHV-8) DNA and antibodies in  
9  
10 4 men seropositive for both HHV-8 and HIV AIDS; 18:1819–1826  
11  
12 5  
13  
14 6 Lebbé C, Blum L, Pellet C, Blanchard G, Vérola O, Morel P, Danne O, Calvo F. 1998.  
15  
16 7 Clinical and biological impact of antiretroviral therapy with protease inhibitors on HIV-  
17  
18 8 related Kaposi's sarcoma. AIDS12: F45-F49.  
19  
20 9  
21  
22  
23  
24 10 Lin L, Lee JY, Kaplan LD, Dezube BJ, Noy A, Krown SE, Levine AM, Yu Y, Hayward GS,  
25  
26 11 Ambinder RF. 2009. Effects of chemotherapy in AIDS-associated non-Hodgkin's lymphoma  
27  
28 12 on Kaposi's sarcoma herpesvirus DNA in blood. J Clin Oncol. May 20;27(15):2496-502  
29  
30  
31  
32 13  
33  
34 14 Lorenzen T, Albrecht D, Paech V, Meyer T, Hoffmann C, Stoehr A, Degen O, Stellbrink HJ,  
35  
36 15 Meigel WN, Arndt R, Plettenberg A. 2002. HHV-8 DNA in blood and the development of  
37  
38 16 HIV-associated Kaposi's sarcoma in the era of HAART--a prospective evaluation. Eur J Med  
39  
40 17 Res; 7:283-6.  
41  
42  
43 18  
44  
45  
46 19 Marcelin AG, Gorin I, Morand P, Ait-Arkoub Z, Deleuze J, Morini JP, Calvez V, Dupin N.  
47  
48 20 2004. .: Quantification of Kaposi's sarcoma-associated herpesvirus in blood, oral mucosa, and  
49  
50 21 saliva in patients with Kaposi's sarcoma. AIDS Res Hum Retroviruses.; 20:704-8.  
51  
52 22  
53  
54  
55 23 Marcelin AG, Motol J, Guihot A, Caumes E, Viard JP, Dussaix E, Cadranel J, Frances C,  
56  
57 24 Carcelain G, Calvez V, Dupin N. 2007.: Relationship between the quantity of Kaposi  
58  
59 25 Sarcoma associated Herpesvirus (KSHV) in peripheral blood and effusion fluid samples and  
60  
26 KSHV associated disease. J Inf Dis,196:1163-6.

- 1  
2  
3 1  
4  
5  
6 2 Martinez V, Caumes E, Gambotti L, Ittah H, Morini JP, Deleuze J, Gorin I, Katlama C,  
7  
8 3 Bricaire F, Dupin N. 2006.: Remission from Kaposi's Sarcoma on HAART is associated with  
9  
10 4 suppression of HIV replication and is independent of protease inhibitor therapy. Br J Cancer  
11  
12 5 94:1000-6  
13  
14 6  
15  
16  
17 7 Moore PS, Kingsley LA, Holmberg SD, Spira T, Gupta P, Hoover DR, Parry JP, Conley LJ,  
18  
19 8 Jaffe HW, Chang Y. 1996. Kaposi's sarcoma-associated herpesvirus infection prior to onset of  
20  
21 9 Kaposi's sarcoma. AIDS 10:175-80.  
22  
23  
24 10  
25  
26  
27 11 Parisi SG, Sarmati L, Pappagallo M, Mazzi R, Carolo G, Farchi F, Nicastrì E, Concia E,  
28  
29 12 Rezza G, Andreoni M. 2002. Prevalence trend and correlates of HHV8 infection among HIV  
30  
31 13 infected individuals. J Acquir Immune Defic Syndr, 29(3), 295-299.  
32  
33  
34 14  
35  
36 15 Parisi SG, Sarmati L, Pappagallo M, Mazzi R, Carolo G, Farchi F, Nicastrì E, Concia E,  
37  
38 16 Rezza G, Andreoni M. 2002. Human herpesvirus 8 cytoviraemia rebound in a patient with  
39  
40 17 Kaposi's sarcoma after a short interruption of efficient antiretroviral therapy. AIDS.  
41  
42 18 16(7):1089-91.  
43  
44  
45 19  
46  
47  
48 20 Parisi SG, Boldrin C, Cruciani M, Nicolini G, Cerbaro I, Manfrin V, Dal Bello F, Franchin E,  
49  
50 21 Franzetti M, Rossi MC, Cattelan AM, Romano L, Zazzi M, Andreoni M,  
51  
52  
53 22 Palù G. 2007. : Both Human Immunodeficiency Virus Cellular DNA Sequencing and Plasma  
54  
55 23 RNA Sequencing are Useful for Detection of Drug Resistance Mutations in Blood Samples  
56  
57 24 from Antiretroviral Drug-Naive Patients. J Clin Microb.; 45(6):1783-1788.  
58  
59  
60 25

- 1  
2  
3 1 Plancoulaine, S., Abel, L., van Beveren, M. & Gessain, A. 2002. High titers of anti-human  
4  
5 2 herpesvirus 8 antibodies in elderly males in an endemic population. *J Natl Cancer Inst*; 94,  
6  
7 3 1333–1335.  
8  
9 4  
10  
11 5 Poole LJ, Zong JC, Ciufu DM, Alcendor DJ, Cannon JS, Ambinder R, Orenstein JM, Reitz  
12  
13 6 MS, Hayward GS. 1999. Comparison of genetic variability at multiple loci across the  
14  
15 7 genomes of the major subtypes of Kaposi's Sarcoma-Associated Herpesvirus reveals evidence  
16  
17 8 for recombination and for two distinct types of open reading frame K15 alleles at the right-  
18  
19 9 hand end. *Journal of Virology* 73: 6646-60.  
20  
21  
22  
23  
24 10  
25  
26 11 Rezza G, Tchangmena OB, Andreoni M, Bugarini R, Toma L, Bakary DK, Glikoutou M,  
27  
28 12 Sarmati L, Monini P, Pezzotti P, Ensoli B. 2000. Prevalence and risk factors for human  
29  
30 13 herpesvirus 8 infection in northern Cameroon. *Sex Transm Dis* 27:159–64.  
31  
32  
33  
34 14  
35  
36 15 Rizzieri DA, Liu J, Traweek ST, Miralles GD. 1997. Clearance of HHV-8 from peripheral  
37  
38 16 blood mononuclear cells with a protease inhibitor [letter]. *Lancet*; 349:775-6.  
39  
40  
41 17  
42  
43 18 Schulz, T. F. & Moore, P. S. 1999. Kaposi's sarcoma-associated herpesvirus: a new human  
44  
45 19 tumor virus, but how? *Trends Microbiol* 7, 196–200.  
46  
47  
48 20  
49  
50 21 Sirianni MC, Vincenzi L, Topino S, Giovannetti A, Mazzetta F, Libi F, Scaramuzzi D,  
51  
52 22 Andreoni M, Pinter E, Baccarini S, Rezza G, Monini P, Ensoli B. 2002. : NK cell activity  
53  
54 23 controls human herpesvirus 8 latent infection and is restored upon highly active antiretroviral  
55  
56 24 therapy in AIDS patients with regressing Kaposi's sarcoma. *Eur J Immunol*. 32(10):2711-20.  
57  
58  
59  
60 25

- 1  
2  
3 1 Smith MS, Bloomer C, Horvat R, Goldstein E, Casparian JM, Chandran B. 1997. Detection of  
4  
5 2 human herpesvirus 8 DNA in Kaposi's sarcoma lesions and peripheral blood of human  
6  
7 3 immunodeficiency virus-positive patients and correlation with serologic measurements. *J*  
8  
9 4 *Infect Dis* 176:84-93.  
10  
11  
12 5  
13  
14 6 Taylor MM, Chohan B, Lavreys L, Hassan W, Huang ML, Corey L, Ashley Morrow R,  
15  
16 7 Richardson BA, Mandaliya K, Ndinya-Achola J, Bwayo J, Kreiss J. 2004.: Shedding of  
17  
18 8 Human Herpesvirus 8 in oral and genital secretions from HIV-1-seropositive and seronegative  
19  
20 9 Kenyan women. *J Inf Dis*,190:484-8.  
21  
22  
23  
24 10  
25  
26 11 Varthakavi V, Smith RM, Deng H, Sun R, Spearman P. 2002. Human immunodeficiency  
27  
28 12 virus type-1 activates lytic cycle replication of Kaposi's sarcoma-associated herpesvirus  
29  
30 13 through induction of KSHV Rta. *Virology* 297: 270–280.  
31  
32  
33  
34 14  
35  
36 15 Viard JP, Burgard M, Hubert JB, Aaron L, Rabian C, Pertuiset N, Lourenço M, Rothschild C,  
37  
38 16 Rouzioux C. 2004. : Impact of 5 years of maximally successful highly active antiretroviral  
39  
40 17 therapy on CD4 cell count and HIV-1 DNA level. *AIDS* 18:45-49.  
41  
42  
43 18  
44  
45 19 Whitby D, Howard MR, Tenant-Flowers M, Brink NS, Copas A, Boshoff C, Hatzioannou  
46  
47 20 T, Suggett FE, Aldam DM, Denton AS. 1995. .: Detection of Kaposi sarcoma associated  
48  
49 21 herpesvirus in peripheral blood of HIV-infected individuals and progression to Kaposi's  
50  
51 22 sarcoma *Lancet*346:799-802.  
52  
53  
54 23  
55  
56 24 White I.E. and Campbell T.B. 2000. Quantitation of Cell-Free and Cell-Associated Kaposi's  
57  
58 25 Sarcoma-Associated Herpesvirus DNA by Real-Time PCR. *Journal of Clinical Microbiology*  
59  
60 26 38 (5):1992-95.

1  
2  
3 1  
4  
5  
6 2 Zeng Y, Zhang X, Huang Z, Cheng L, Yao S, Qin D, Chen X, Tang Q, Lv Z, Zhang L, Lu C.  
7  
8 3 2007. . Intracellular Tat of human immunodeficiency virus type 1 activates lytic cycle  
9  
10 4 replication of Kaposi's sarcoma-associated herpesvirus: role of JAK/STAT signaling. J Virol  
11  
12 5 81: 2401–2417.  
13  
14  
15 6  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Peer Review