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Different Impact of Anti-retroviral Regimen Containing Protease Inhibitors on Development of HIV-related Kaposi Sarcoma

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Abstract. *Background: The incidence of Kaposi's sarcoma (KS), an AIDS-related malignancy, has dramatically decreased in the Highly Active Anti-retroviral Therapy (HAART) era. However, KS remains the second most frequent tumor in HIV-infected patients worldwide and has become the most common cancer in the sub-Saharan Africa. Experimental studies have demonstrated a direct anti-neoplastic effect of HAART, and overall of protease inhibitors (PIs), on KS. Case Report: We describe five cases of KS in HIV-infected patients on HAART regimen, containing PIs as atazanavir/r (ATV/r), darunavir/r (DRV/r), lopinavir/r (LPV/r) and fosamprenavir (fAMP/r). Conclusion: Clinical and experimental observations support the hypothesis that PIs may play an important role in prevention and treatment of KS. In our study, the treatment with PIs of recent generation was not protective against the development of KS. Therefore, it could be necessary to re-evaluate the therapeutic effects of PIs and their role in the development and treatment of KS in HIV-infected patients.*

Kaposi's sarcoma (KS) is a multi-focal angioproliferative disease of endothelial origin, characterized by the presence of multiple purplish-red pigmented nodular lesions, usually involving cutaneous and mucocutaneous sites. The pathogenesis of KS represents a complex of events where the interaction of immunosuppression, altered cytokine production, the action of Human Immunodeficiency Virus (HIV) and Human Herpes Virus 8 (HHV-8) are involved (1). In HIV-infected patients, decreased $CD4^+$ lymphocyte counts, altered $CD4^+/CD8^+$ ratio, high viral load and absence of Highly Active Anti-retroviral Therapy (HAART) regimen are independent factors associated with the development of KS, as Acquired Immune Deficiency Syndrome (AIDS) defining illness (1, 2).

KS was the commonest HIV-1-related malignancy before HAART introduction. In Western countries, the widespread introduction of HAART in HIV-infected patients has been associated with a marked reduction of KS incidence, an extended time to treatment failure and KS resolution (1, 3). However, KS remains the second most frequent tumor in HIV-infected patients worldwide and it has become the most common cancer in sub-Saharan Africa, where the burden of HIV and HHV-8 co-infection is high and HAART is not widely available (1). The efficacy of HAART on KS was described for the first time in 1997 in several anecdotal reports and then in some retrospective studies (3).

In 1998, Lebbè *et al*. analyzed a cohort of 10 HIV-infected patients with stable or progressive KS. Patients received an antiviral therapy including protease inhibitors (PIs) as indinavir (IDV), ritonavir (RTV) and saquinavir (SQV), with an 80% response to therapy, independently of previous treatments or chemotherapy (4). These data were confirmed by Cattelan *et al*. in another similar prospective study, where 11 patients, affected by both HIV infection and KS, were treated with a combination of a PI, as IDV, RTV, SQV and nelfinavir (NLF) with a backbone of two nucleoside reverse-transcriptase inhibitors (NRTI) (5). The authors concluded that the complete response was associated with the restoration of immunity and a decrease of both HIV plasma viral load and HHV-8 viremia in the peripheral blood mononuclear cells (3-5). Another possible mechanism for the association between KS regression and effective anti-

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retroviral therapy is the inhibition of Tat protein synthesis resulting in down-regulation of several related proliferative pathways such as the growth of KS-derived spindle cells, the angiogenesis process and the induction of a number of cytokines (3-5).

The specific role of PIs in the resolution of KS is also demonstrated by experimental studies in various oncological contests (6-11). It has been reported that the systemic administration of IDV and SQV *in vitro* and *in vivo* to nude mice is able to block the development and to induce regression of angioproliferative KS-like lesions established by primary human KS cells, by the inhibition of matrix metalloproteinase-2 proteolytic activation (6).

In addition Pati *et al*. demonstrated *in vitro* inhibition of activation and proliferation of primary endothelial cells, decreased production of tumor necrosis factor alpha, interleukins -6 and -8, and VEGF by RTV. This last drug was also shown to inhibit tumor formation and progression by KS-derived cells in a KS mouse xenotransplantation model (7). Similarly, Spugnini *et al*. evaluated the efficacy of IDV in a canine hemangiosarcoma model (8) and Esposito *et al*. reported the anti-tumor effects of IDV (9) and amprenavir (AMP) (10) on hepatocarcinoma cell lines *in vitro* and *in vivo*.

According to clinical and experimental results, PIs seem to have direct anti-KS and/or anti-angiogenic abilities, because they can directly interfere with angiogenic and inflammatory processes and with tumor growth, independently of the immune response. These studies led to the suggestion that a PIs containing HAART regimen might be more effective than other effective anti-retroviral regimen in preventing KS (1, 6).

Despite literature data demonstrating that HAART either alone or in combination with systemic and local therapy plays a crucial role in prevention and control of KS, we observed five cases of KS in HIV-infected patients on PIs containing HAART regimen with well-controlled HIV infection.

Case Report

We observed five cases of KS in HIV-1-infected patients, admitted to the Third and the Fifth Unit of Infectious Diseases of "D. Cotugno" Hospital of Naples between 2007 and 2012. Patients were all young adult males and injective-drug users. Four were smokers and three had HIV-1 and Hepatitis C Virus (HCV) co-infection. The HIV stage CDC (Centers for Disease Control) was variable, as well as the prescribed HAART regimens (Table I). All five patients reached a good viro-immunologic control ($CD4^+ > 300/mm^3$ and undetectable HIV RNA), but one, who presented HIV RNA 313,400 cps/ml; the same patient was affected by both cutaneous and visceral KS, while the other four patients presented only cutaneous lesions. At the moment of KS diagnosis, all five patients followed HAART regimens containing PIs of relatively recent development such as lopinavir/r (LPV/r), atazanavir/r (ATV/r), fosamprenavir (fAMP/r) and darunavir (DRV/r). Three of them continued the same HAART regimen in combination with systemic chemotherapy. Clinical, laboratoristic and therapeutic features and outcome of the five reported patients are presented in Table I.

Discussion

We herein described five cases of KS in HIV-1-infected patients developed during HAART regimen containing PIs of recent generation such as fAMP/r, LPV/r, ATV/r, DRV/r. Four patients showed a strict adherence to the anti-retroviral therapy, including LPV/r, ATV/r and fAMP/r, with the result of a progressive increase of CD4 T-cell count and undetectability of HIV RNA levels. The fifth patient referred a poor adherence to HAART regimen containing DRV/r, demonstrated by the high HIV viral load (>100,000 cps/ml), despite his satisfactory immunological control (CD4 $>300/mm^3$).

According to literature data, the state of immuno-suppression reduces tumor surveillance and increases the risk of neoplasm; especially, the development of KS is correlated to the decrease of the CD4 T cell count, the high viral load and the absence of HAART regimen. The anti-retroviral treatment is also reported to be more effective if it restores or maintains the CD4 T-cell count $>500/mm^3$ (2). In addition, it is demonstrated that HAART decreases immune activation and cytokine levels, boosts immune responses unmeasured by the CD4 cell count, and suppresses the oncogenic viruses (12). Therefore, HAART has a protective immune-modulating effect that should reduce the risk of KS outbreak.

According to our case reports, recent observational studies demonstrated an increased proportion of cases of KS in subjects with effective HIV suppression and CD4 T cell count $>200/mm^3$ (13-17). In particular, Maurer *et al*. described a cluster of unusual cases of cutaneous, unremitting HIV-associated KS occurring in nine patients with CD4 T cell count >300/mmc and undetectable HIV viral load for at least 2 years. All patients were on treatment with anti-retroviral regimens containing at least one PI or non-nucleoside reverse-transcriptase inhibitor (NNRTI) (13). Similarly, Known *et al*. from the AIDS Malignancy Consortium, analyzing a cohort of 442 patients affected by KS, suggested that persistence of this malignancy despite apparently effective antiretroviral therapy is not a rare isolated or recent phenomenon (14). In this light it could be relevant to investigate other mechanisms that could explain the pathogenesis and the evolution of KS.

Table I. *Clinical and laboratory features of five HIV-infected patients with KS.*

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Gender	Male	Male	Male	Male	Male
Age at HIV diagnosis	22	35	35	30	45
Risk factors	Injecting-drug user	Injecting-drug user	Injecting-drug user	Injecting-drug user	Injecting-drug user
Smoker	No	Yes	Yes	Yes	Yes
Comorbidities	Systemic hypertension	Cardiovascular disease; HCV co-infection	none	HCV co-infection	HCV co-infection
First HIV diagnosis	1991	2007	1999	2003	1998
Stage CDC	C3	A3	A2	A1	A3
CD4+ (cells/mmc) at HIV onset	199	111	224	676	338
HIV RNA (cps/ml) at HIV onset	Not available	30,871	205,838	74,700	1,152,910
nadir CD4+ (cells/mmc)	5	111	224	578	158
Previous HAART regimens	ddC+AZT 3TC+ ddI IDV+3TC+d4T LPV+AMP+ddI TDF+LPV/r	TDF+FTC+ ATV/r	AZT+3TC+NFV AZT+3TC+LPV/r TDF+FTC+EFV TDF+FTC+fAMP/r	TDF+FTC+ fAMP/r	AZT+ddI+RTV AZT+3TC+IDV TDF+FTC+INN TDF+FTC+ DVR600/r
HIV drug-resistance profile	No mutation	No mutation	No mutation	No mutation	24I, 35D, 46IM, 54V, 63P, 82A, 67N, 70R, 82A, 135M, 184V, 211K, 219Q
Year KS diagnosis	2009	2009	2007	2008	2010
KS localization	Cutaneous	Cutaneous	Cutaneous	Cutaneous	Cutaneous and visceral (stomach)
CD4+ (cells/mmc) at KS diagnosis	748	462	362	578	333
HIV RNA (cps/ml) at KS diagnosis	<40	<40	<40	<40	313,400
Time of viral suppression before KS diagnosis	8 years	2 years	2 years	5 years	No viral suppression
HAART regimen at KS diagnosis	TDF+LPV/r	TDF+FTC+ ATV/r	TDF+FTC+ fAMP/r	TDF+FTC+ fAMP/r	TDF+FTC+ DVR600/r
KS evolution	Stable	Spontaneous remission	Remission	Remission	Partial remission
HAART change	None	None	None	None	TDF+FTC+ DVR600/r+RGV
Chemotherapy or other treatment	None	None	Doxorubicin	Doxorubicin	Doxorubicin

ddC, Zalcitabine; AZT, zidovudine; 3TC, lamivudine; ddI, didanosine; IDV, indinavir; d4T, stavudine; LPV, lopinavir; AMP, amprenavir; TDF, tenofovir; LPV/r, lopinavir/r; FTC, emtricitabine; ATV/r, atazanavir/r; NFV, nelfinavir; EFV, efavirenz; fAMP/r, fosamprenavir/r; RTV, ritonavir; INN, tipranavir; DVR/r, darunavir/r; RGV, raltegravir.

The previously described observational studies reported the outbreak of KS during different HAART regimens including PIs or NNRTI. None of these regimens has been found to be more effective in the prevention of KS (1, 13-16, 18), but *in vitro* models support a direct effect only of first generation anti-retroviral agents such as SQV, IDV, NFV and RTV on angiogenesis and cell invasion, promotion of apoptosis on HHV-8 replication and production of HIV-1 Tat (6-9, 11). These data highlight the concept that PIs may play an important role in the prevention and treatment of KS with a different efficacy, dependent to different molecules (2). Therefore, comparative studies on the potential anti-neoplastic and anti-angiogenic activities of more recent PIs are urgently required. In fact, we observed, in accordance to previous reports, that the treatment with HAART regimen including PIs of recent generation (fAMP/r, LPV/r, ATV/r, DRV/r) was not protective against the outbreak of KS. Due to the heterogeneity of the treatment regimens prescribed and the limited number of subjects, we were unable to definitively assess the effects of these PIs on KS development.

References

1 La Ferla L, Pinzone MR, Nunnari G, Martellotta F, Lleshi A, Tirelli U, De Paoli P, Berretta M and Cacopardo B: Kaposi's sarcoma in HIV-positive patients: the state of art in the HAART-era. Eur Rev Med Pharmacol Sci *17*: 2354-2365, 2013.

2 Guiguet M, Bouè F, Cadranel J, Lang, JM, Rosenthal E, Costagliola D, on behalf of the Clinical Epidemiology Group of the FHDH-ANRS CO4 cohort: Effect of immunodeficiency, HIV viral load, and antiretroviral therapy on the risk of individual malignancies (FHDH-ANRS CO4): a prospective cohort study. Lancet Oncol *10*: 1152-59, 2009.

3 Aversa SML, Cattelan AM, Salvagno L, Crivellari G, Banna G, Trevenzoli M, Chiarion-Sileni V and Monfardini S: Treatments of AIDS-related Kaposi's sarcoma. Crit Rev Oncol Hematol *53*: 253-265, 2005.

4 Lebbè C, Blum L, Pellet C, Blanchard G, Vèrola O, Morel P, Danne O and Calvo F: Clinical and biological impact of antiretroviral therapy with protease inhibitors on HIV-related Kaposi's sarcoma. AIDS *12*: F45-F49, 1998.

5 Cattelan AM, Calabrò ML, Aversa SML, Zanchetta M, Meneghetti F, De Rossi A and Chieco-Bianchi L: Regression of AIDS-related Kaposi's sarcoma following antiretroviral therapy with protease inhibitors: biological correlates of clinical outcome. Eur J Cancer *35*: 1809-1815, 1999.

6 Sgadari C, Barillari G, Toschi E, Carlei D, Bacigalupo I, Baccarini S, Palladino C, Leone P, Bugarini R, Malavasi L, Cafaro A, Falchi M, Valdembri D, Rezza G, Bussolino F, Monini P and Ensoli B: HIV protease inhibitors are potent anti-angiogenic molecules and promote regression of Kaposi sarcoma. Nat Med *8*: 225-232, 2002.

7 Pati S, Pelser CB, Dufraine J, Bryant JL, Reitz MS Jr., Weichol FF: Antitumorogenic effects of HIV protease inhibitor ritonavir: inhibition of Kaposi sarcoma. Blood *99*: 3771-3779, 2002.

8 Spugnini EP, Esposito V, Groeger AM, Cassandro R, Onori N, Chirianni A and Baldi A: Effects of indinavir in a preliminary phase I study on dogs with stage III splenic hemangiosarcoma. In Vivo 20: 125-128, 2006.

9 Esposito V, Palescandolo E, Spugnini EP, Montesarchio V, De Luca A, Cardillo I, Cortese G, Baldi A and Chirianni A: Evaluation of antitumoral properties of the protease inhibitor indinavir in a murine model of epatocarcinoma. Clin Cancer Res *12*: 2634-2639, 2006.

10 Esposito V, Verdina A, Manente L, Spugnini EP, Viglietti R, Parrella R, Pagliano P, Parrella G, Galati R, De Luca A, Baldi A, Montesarchio V and Chirianni A: Amprenavir inhibits the migration in human hepatocarcinoma cell and the growth of xenografts. J Cell Physiol *228*: 640-645, 2013.

11 Gantt S, Casper C and Ambinder RF: Insight into the broad cellular effects of nelfinavir and the HIV protease inhibitors supporting their role in cancer treatment and prevention. Curr Opin Oncol *25*: 495-502, 2013.

12 Crum-Cianflone N, Hullsiek KH, Marconi V, Weintrob A, Ganesan A, Barthel RV, Fraser S, Agan BK and Wegner S: Trends in the incidence of cancers among HIV-infected persons and the impact of antiretroviral therapy: a 20-year cohort study. AIDS *23*: 41-50, 2009.

13 Maurer T, Ponte M and Leslie K: HIV-associated Kaposi's sarcoma with a high CD4 count and a low viral load. N Engl J Med *357*: 1352-1353, 2007.

14 Known SE, Lee JY, Dittmer DP and AIDS Malignancy Consortium: More on HIV-associated Kaposi's sarcoma. N Engl J Med *358*: 535-536, 2008.

15 Mani D, Neil N, Israel R and Aboulafia DM: A retrospective analysis of AIDS-associated Kaposi's sarcoma in patients with undetectable HIV viral loads and CD4 counts greater than 300 cells/mm(3). J Int Assoc Physicians AIDS Care (Chic) *8*: 279-285, 2009.

16 Maurer T, Leslie K and Unemori P: Response to: A retrospective analysis of AIDS-associated Kaposi's sarcoma in patients with undetectable HIV viral loads and CD4 counts greater than 300 cells/mm3. J Int Assoc Physicians AIDS Care *9*: 73, 2010.

17 Crum-Cianflone N, Hullsiek KH, Ganesan A, Weintrob A, Okulicz J, Agan BK and the Infectious Disease Clinical Research Program HIV Working Group: Is Kaposi's sarcoma occurring at higher CD4 counts over the course of the HIV epidemic? AIDS *24*: 2881-2883, 2010.

18 Nguyen HQ, Magaret AS, Kitahata MM, Van Rompaey SE, Wald A and Casper C: Persistent Kaposi sarcoma in the era of HAART: characterizing the predictors of clinical response. AIDS *22*: 937-945, 2008.

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