Use of HIV protease inhibitors to block Kaposi's sarcoma and tumour growth

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HIV protease inhibitors are antiretroviral drugs that block the enzyme required for production of infectious viral particles. Although these agents have been designed to selectively bind to the catalytic site of HIV protease, evidence indicates that other cellular and microbial enzymes and pathways are also affected. It has been reported that patients treated with highly active anti-retroviral therapy (HAART) containing a protease inhibitor may be at reduced risk of Kaposi's sarcoma (KS) and some types of non-Hodgkin lymphomas; some disease regressions have also been described. Here we review recent data showing that several widely used protease inhibitors, including indinavir, saquinavir, ritonavir, and nelfinavir, can affect important cellular and tissue processes such as angiogenesis, tumour growth and invasion, inflammation, antigen processing and presentation, cell survival, and tissue remodelling. Most of these non-HIV-related effects of protease inhibitors are due to inhibition of cell invasion and matrix metalloprotease activity, or modulation of the cell proteasome and NFκB. These elements are required for development of most tumours. Thus, by direct and indirect activities, protease inhibitors can simultaneously block several pathways involved in tumour growth, invasion, and metastasis. These findings indicate that protease inhibitors can be exploited for the therapy of KS and other tumours that occur in both HIV-infected and non-infected individuals. A multicentre phase II clinical trial with indinavir in non-HIV-associated KS is about to start in Italy.

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The advent of new antiretroviral drugs, most notably HIV protease inhibitors, has generated new hope in the fight against AIDS. Used in combination with nucleoside inhibitors of HIV reverse transcriptase, protease inhibitors have led to impressive clinical outcomes. Such combined therapeutic regimens, known as highly active antiretroviral therapies (HAART), work by suppressing HIV replication and can lead to a large reduction in HIV plasma viraemia, restoration of normal numbers of CD4-positive T lymphocytes, immunological recovery, and reduction of morbidity and mortality related to HIV and opportunistic infections.[1] The increase in CD4-positive T-cell counts and the immune restoration that occurs with HAART is most likely to depend on the following mechanisms: increased peripheral CD4-positive T-cell survival and proliferation, central renewal of lymphocytes, improvement of T-cell responses, and restoration of the T-cell repertoire.[2] Therefore, protease-inhibitor-based HAART owes its success to the ability to block HIV replication and promote subsequent immunological recovery. Unexpectedly, however, in some individuals who have a good clinical response to HAART, and a remarkable increase of CD4-positive T cells, HIV viraemia is not controlled.[2] Conversely, some patients that respond to HAART show little or no recovery of CD4-positive T-cell counts despite a decline in HIV load.[2] Growing evidence indicates that protease inhibitors may act against HIV infection through additional mechanisms that are unrelated to their specific antiretroviral activity.

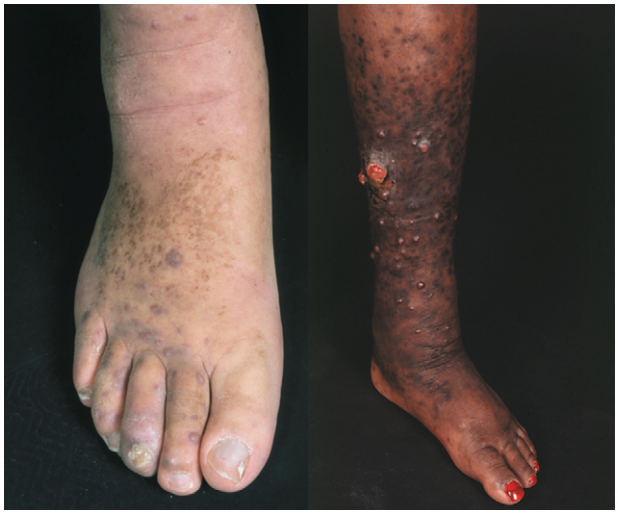

Figure 1. Different types of Kaposi's sarcoma. Immunosuppression KS (left). Endemic African KS (right).

Protease inhibitors mimic endogenous peptides and thereby block the active site of HIV aspartyl protease, a retroviral enzyme that cleaves the viral gag-pol polyprotein; this action prevents production of infectious viral particles.[1] Although protease inhibitors have been designed to selectively block the HIV protease catalytic site,[1] long-lasting administration of HAART causes unpredicted adverse effects including hyperbilirubinaemia, insulin resistance and diabetes, hyperlipidaemia or hypolipidaemia, cardiovascular diseases, body fat redistribution, osteopenia, and osteoporosis.[3] Some of these adverse effects are due to actions on proteins involved in important metabolic pathways, in

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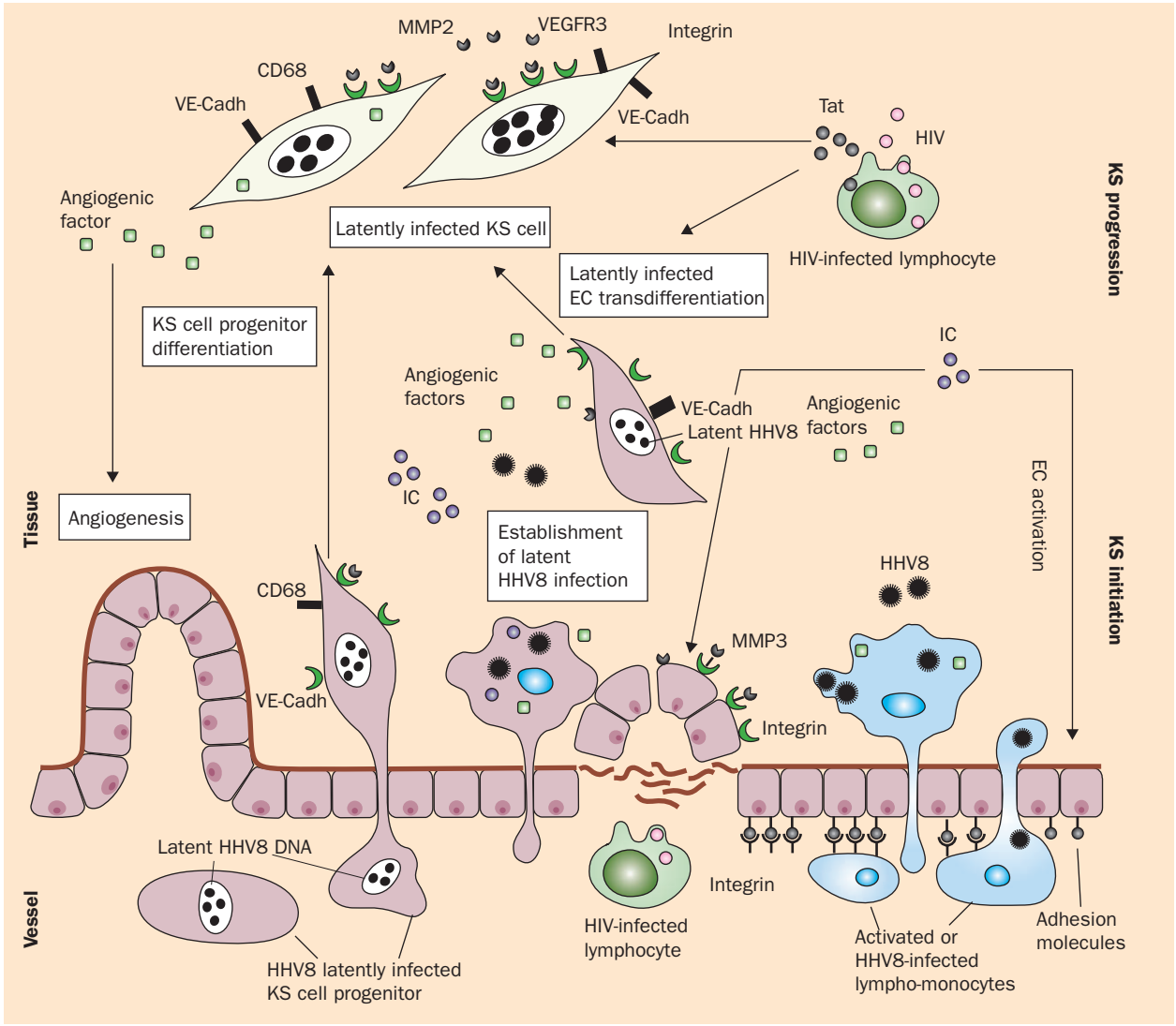

Figure 2. Individuals with HHV8 infection and a dysregulation of the immune system are at risk of developing Kaposi's sarcoma. The key factors that lead to tumour initiation and progression are summarised here. VE-Cadh, VE-cadherin; MMP, matrix metalloproteinase; VEGFR, vascular endothelial growth factor receptor; EC, endothelial cell; IC, inflammatory cytokines; HHV, human herpesvirus.

addition to effects on adipocytes, osteoclast and osteoblast function, and differentiation.[4–7] For example, ritonavir and saquinavir affect degradation and secretion of apolipoprotein B, whereas indinavir inhibits bilirubin UDP-glucoronosyl-transferase and the glucose transporter Glut4.[4–7] In addition, nelfinavir and lopinavir decrease osteoprotegrin and osteoblast alkalin phosphatase expression,[4–6] and indinavir, nelfinavir, and ritonavir can perturb production and subcellular localisation of key adipogenic transcription factors including C/EBPα, PPARγ, or SREBP1 and SREBP2.[8–11] However, although indinavir and nelfinavir inhibit the expression and the nuclear import of all these factors, which leads to a blockade of preadipocyte differentiation and to adipose tissue atrophy, ritonavir has opposite effects on SREBP1 and SREBP2 that are likely to be involved in adipose accumulation, insulin resistance, and diabetes.[8–11] However, in addition to these adverse effects, protease inhibitors have other actions that might be beneficial both for HIV infection and other diseases. In particular, ritonavir and saquinavir have been shown to affect important cellular pathways and responses including antigen processing and presentation, NFκB activity,[12–15] and production or release of inflammatory cytokines and chemokines (eg, TNFα, interleukin 6, and interleukin 8) by peripheral blood mononuclear cells (PBMC) or endothelial cells.[15,16] Specifically, ritonavir can inhibit the activation of NFκB induced by TNFα, human herpesvirus 8 (HHV8), or HIV-1 Tat, which results in apoptosis.[15] Ritonavir, indinavir, saquinavir, and nelfinavir can all block activation of endothelial cells and T cells, maturation and function of dendritic cells, and can modulate proliferation, apoptosis, or differentiation of PBMCs, T cells, adipocytes, osteoblasts and osteoclasts, and myelocytic leukaemia cells.[8–10,15–22] Increasing evidence indicates that several of these actions are most likely to be mediated by the effects of protease inhibitors on the cell proteasome.[12–15] However, other pathways also seem to be involved. In fact, although proteasomal activity, and T-cell

survival and proliferation, are all inhibited at drug concentrations similar to or above the therapeutic concentrations in treated patients (5–10 μM), T-cell proliferation and survival are strongly stimulated at much lower concentrations (1–100 nM); these amounts are similar to the lowest concentrations found in treated patients and do not affect proteasomal activity.[13,15–23] In addition to this it has been shown that indinavir, ritonavir or saquinavir can directly block aspartyl proteases in *Candida albicans* and *Pneumocystiis carinii*, which explains at least in part the reduced morbidity and mortality of these opportunistic infections in patients treated with protease inhibitors.[24,25]

All the effects of protease inhibitors—which have been proven in HIV-free, controlled experimental systems—may actually increase the therapeutic effectiveness of HAART in HIV-infected individuals by directly reducing uncontrolled immune activation, inflammation, T-cell apoptosis, and opportunistic infections, or by helping to restore T-cell proliferative responses.[19] Of particular note, in people who received protease-inhibitor-based HAART as prophylactic therapy without acquiring HIV infection, PBMCs produced smaller amounts of inflammatory cytokines including TNFα, interferon γ, and interleukin 2, indicating that the in-vivo effects of protease inhibitors may not be mediated by suppression of HIV replication.[22]

Use of HAART has been associated with a substantial reduction in the incidence of HIV-associated malignant diseases including KS and some non-Hodgkin lymphomas (cerebral and immunoblastic lymphomas).[26,27] This antitumour effect is underlined by the frequent regression of KS in patients treated with HAART and by the decreased frequency of extranodal lymphoma localisations.[19,28–30] In addition, anecdotal regressions of primary effusion lymphomas and cervical intraepithelial neoplasia have also been reported.[31,32] Since these tumours are associated with infection by HHV8, Epstein-Barr virus, and human papillomaviruses, the antitumour effect of protease inhibitors has been interpreted as a consequence of the immunological reconstitution induced by HAART. Notably, however, HAART does not seem to interfere with the incidence of other cancers associated with viral infections, such as Burkitt's and Hodgkin's lymphomas, which can develop at higher numbers of CD4-positive T cells in patients with HIV.[26] In addition, it has not yet been possible to establish correlation between KS regression and HIV suppression or the recovery of CD4-positive T cells in treated patients.[33,34] Thus, although a longer follow-up is needed for definitive conclusions to be drawn, these data support the hypothesis that reduced incidence or regression of KS and NHL in patients treated with HAART may be attributable, at least in part, to direct antitumour effects of protease inhibitors. However, the specific mechanisms by which these drugs exert their antitumour effects remain unclear.

Inhibition of KS by protease inhibitors

The most impressive antitumour effects of protease inhibitors are, undoubtedly, the regression and the reduced incidence of KS in patients treated with HAART containing protease inhibitors.[26,28,30] KS is an angioproliferative disease characterised by inflammatory cell infiltration, intense and aberrant angiogenesis, oedema, and growth of spindle cells of endothelial or monocytic cell origin (KS cells); these cells express markers such as CD68 and VE-cadherin.[35] Four different clinical-epidemiological forms of KS have been described: classical KS, which sporadically occurs in elderly men of Mediterranean origin; post-transplant KS, that can arise in recipients of organ transplants; AIDS-associated KS (AIDS-KS), the most frequent tumour of individuals infected by HIV-1; and African KS, which is endemic in subequatorial Africa.[35,36]

KS development is a multistep process involving several factors including infection by HHV8,[27,37] production of inflammatory cytokines and angiogenic factors[35,36] and, in patients infected with HIV, by actions of the HIV-1 Tat protein,[38] which has an important role in disease onset and progression. Earlier studies have shown that PBMC-associated HHV8 viraemia or high antibody titres against HHV8-latency-associated antigens or lytic antigens are predictive of KS onset in people infected with HIV-1.[39–42] However, the progression rate to KS is significantly lower in individuals with HHV8 seroconversion before HIV-1 infection than in HIV-1-infected individuals.[43,40] A higher HHV8 seroprevalence is observed in Mediterranean countries or African regions where KS is frequent or endemic, although in these regions HHV8 infection is much more common than cases of KS.[35,36] These findings indicate that HHV8 is necessary, but not sufficient, for KS development. In fact, although KS is rare in elderly people of Mediterranean origin and in patients who receive transplants in the absence of HIV infection, its incidence and aggressiveness are dramatically increased in patients with HIV infection. This is due to the actions of inflammatory cytokines, particularly interferon γ, interleukin 1β, and TNFα, which are all present in increased concentrations in HIV-infected people and other individuals at risk of KS. These inflammatory cytokines reactivate HHV8[44,45] (leading to virus dissemination

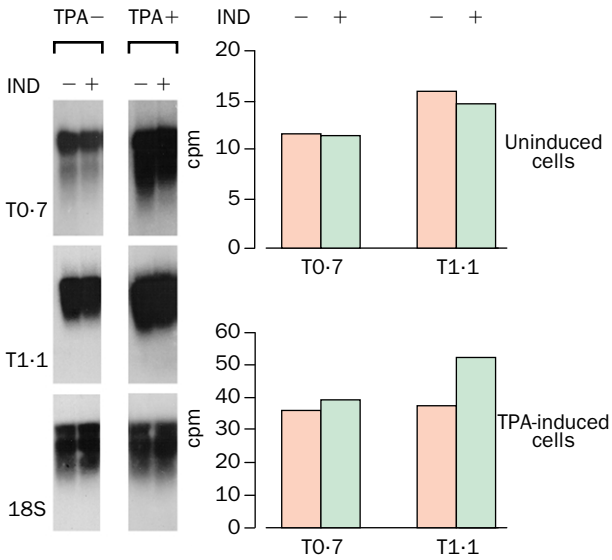

Figure 3. Indinavir (IND) does not inhibit lytic (T1.1) and latent (T0.7) HHV8 gene expression in primary effusion lymphoma cells. Left panel: northern blot autoradiogram; right panel: quantification of hybridisation bands. Virus gene expression was normalised to 18S RNA.

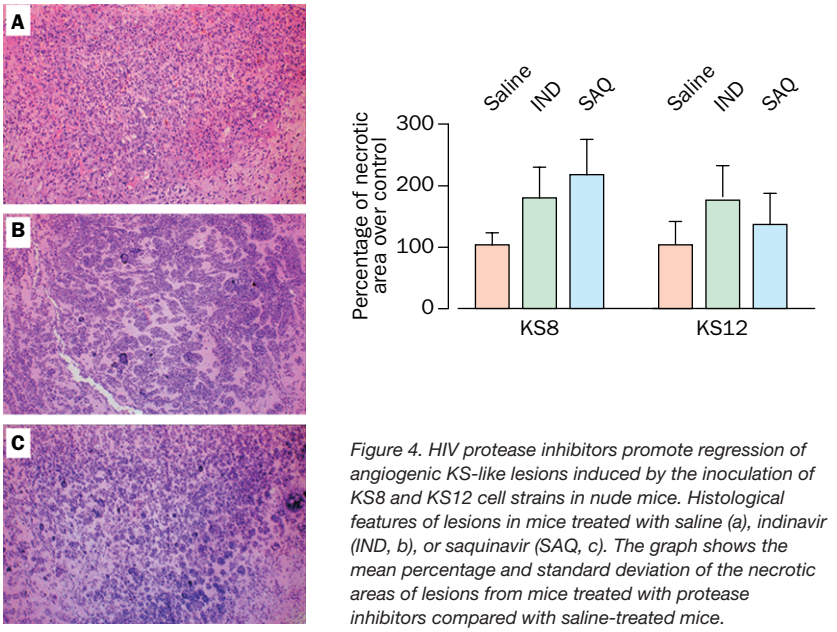

Figure 4. HIV protease inhibitors promote regression of angiogenic KS-like lesions induced by the inoculation of KS8 and KS12 cell strains in nude mice. Histological features of lesions in mice treated with saline (a), indinavir (IND, b), or saquinavir (SAQ, c). The graph shows the mean percentage and standard deviation of the necrotic areas of lesions from mice treated with protease inhibitors compared with saline-treated mice.

to blood cells) and activate vessels,[44–46] which promotes extravasation into tissues of activated lymphocytes and monocytes, a fraction of which are infected by the virus. These processes lead to HHV8 dissemination to tissue cells including KS cells and, in turn, induce immune responses against the virus. However, in individuals at risk of KS, the immune reaction is ineffective and, paradoxically, acts to exacerbate the reactive process and production of inflammatory cytokines (figure 2).[35] Thus, evidence indicates that progenitors of KS cells that are present in blood of patients with KS, or those at risk of KS, are also recruited into tissues.[35] These cells are latently infected by HHV8, transmigrate from the bloodstream through the activated endothelia, and differentiate in latently infected spindle cells that localise in lesions and express host and HHV8 antiapoptotic genes.[35] This is believed to lead to the multifocal appearance and growth of KS lesions at independent sites (figure 2).[35]

The increase in expression by infiltrating cells of inflammatory cytokines and the consequent increase in systemic concentrations of these substances induces production of angiogenic factors, particularly basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), which are potent angiogenic compounds highly expressed in KS and, notably, in most tumours.[35,38,47–49] Several in-vitro and in-vivo studies have shown that bFGF and VEGF have a key role in KS development. In particular, bFGF is an autocrine mediator of growth of primary KS cells that are derived from human lesions and, synergistically with VEGF, promotes development of murine angioproliferative KS-like lesions induced by inoculation of KS cells in nude mice.[38,44,45,48–54] In fact, inoculation of mice with bFGF and VEGF and their in-vivo induction with inflammatory cytokines leads to angiogenesis, spindle-cell growth, and formation of vascular lesions.[38,46,49,54–56] Finally, bFGF acts synergistically with HIV-1 Tat, which is released by HIV-1 infected cells, to increase the frequency and aggressiveness of KS in HIV-1 infected individuals.[38,56–60] This happens because Tat can mimic extracellular matrix proteins by binding to $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins, which are induced by inflammatory cytokines and bFGF (figure 2).[38,55,57,61]

Of particular note, the most important KS traits—tissue infiltration by cells producing inflammatory cytokines, angiogenesis, KS cell dissemination, and lesion growth—are all dependent on the capability of cells to invade the basement membrane and to migrate in interstitial tissue upon degradation of the extracellular matrix. In KS, most solid tumours, and in angiogenesis, this process is mediated by specific matrix metalloproteases (MMPs) and in particular by MMP2, which is highly expressed in all forms of KS and is induced by bFGF.[60]

Therefore, there are several key factors involved in KS pathogenesis that may be the target of protease inhibitor activity (table). Inhibition of HIV replication with HAART leads to reconstitution of the immune system and regenerates effective immune responses against both HHV8 and KS cells.[62–64] This also leads to a decrease in the production and release of HIV-1 Tat, which, as mentioned before, acts as a progression factor for KS.[52,55–59,65] Furthermore, protease inhibitors block the production and synthesis of inflammatory cytokines such as interferon γ, TNFα, interleukin 1α, and interleukin 2 in activated PBMC, endothelial cells, and lymphoid tissues, even in the absence of HIV infection.[15,16,22,66] This, in turn, is likely to result in down-regulation of bFGF and VEGF production, and of HHV8 reactivation, which are all induced by inflammatory cytokines.[35,36,44–46,49,55–57,67] In fact,both VEGF and bFGF and HHV8 load are decreased in serum samples from patients who respond to HAART and/or who show KS regression (Ensoli B,

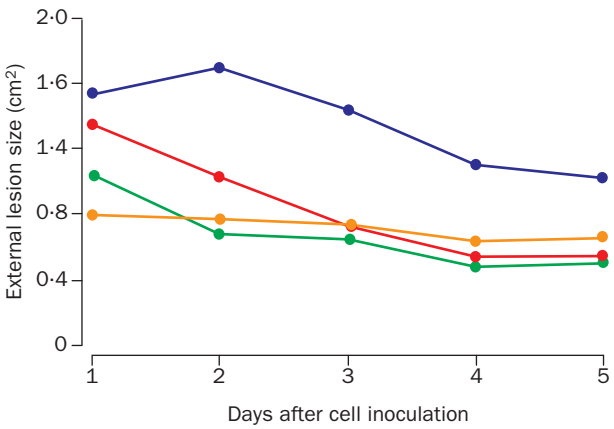

Figure 5. Prostate inhibitors promote the regression of angiogenic KS-like lesions induced by the inoculation of KS cells in nude mice in the absence of pretreatment. The mean external lesional area present in untreated (blue), indinavir (red) or saquinavir (green) treated mice injected with KS cells, or in the negative controls (orange).

unpublished data).[28,35,36,68–71] These mechanisms are likely to play a part in patients with KS, or at risk of KS, who have been treated with HAART. However, recent studies indicated that the KS inhibition observed in treated patients does not necessarily correlate with immune reconstitution nor with HIV or HHV8 suppression.[33,34,72] In addition, no significant KS regression was observed before the HAART era, despite the fact that conventional antiretroviral therapies were successful in blocking HIV replication.[73,74] In a recent prospective study, patients with AIDS-associated KS treated with HAART containing either a protease inhibitor or the novel non-nucleoside reverse transcriptase inhibitors (NNRTI) showed a similar reduction in HIV and HHV8 load, but complete KS regression was only observed in patients who received the protease inhibitor.[75]

Thus, additional effects are likely to be involved in the reduced incidence and regression of KS after treatment with HAART. Notably, these effects are not related to a direct action of protease inhibitors on HHV8 infection. We have not detected any direct inhibition of HHV8 latent infection or HHV8 reactivation by several widely used protease inhibitors including indinavir, saquinavir (figure 3), and nelfinavir.[76] This implies that protease inhibitors have direct antitumour effects.

Protease inhibitors directly affect KS

Although the potential direct effects of protease inhibitors on KS are masked by HIV suppression and by the reconstitution of the immune system after treatment with HAART, these can be studied using in-vivo models of KS formation in the absence of viral agents and T cells; one example of such a model is the KS-like lesions induced in athymic nude mice by the subcutaneous inoculation of human primary KS cells derived from KS lesions.[29,38,44,52,77] These cells lose HHV8 when they are cultured and are free of HIV or other viruses.[35] This model has already been established as a test for preclinical efficacy of therapies against KS.[38,77–79] As discussed above, the angioproliferative lesions induced in nude mice by KS cells closely resemble early human KS. In fact, these lesions develop in response to cytokines such as bFGF and VEGF, which are released by KS cells and are characterised by intense neoangiogenesis, spindle-cell proliferation, and oedema.[35,44–46,49,51,52] To verify the effects of two widely used protease inhibitors in KS, we used intragastric gavage to treat nude mice with indinavir or saquinavir. At doses similar to those administered to treated patients, both indinavir and saquinavir significantly reduced the number and size of macroscopic KS-like angioproliferative lesions developed at the injection site.[29] Microscopic examination of the lesions showed that protease-inhibitor treatment promoted the formation of a large central necrotic area involving up to 85% of the whole tumour (figure 4); there was also a marked reduction in new vessel formation, oedema,

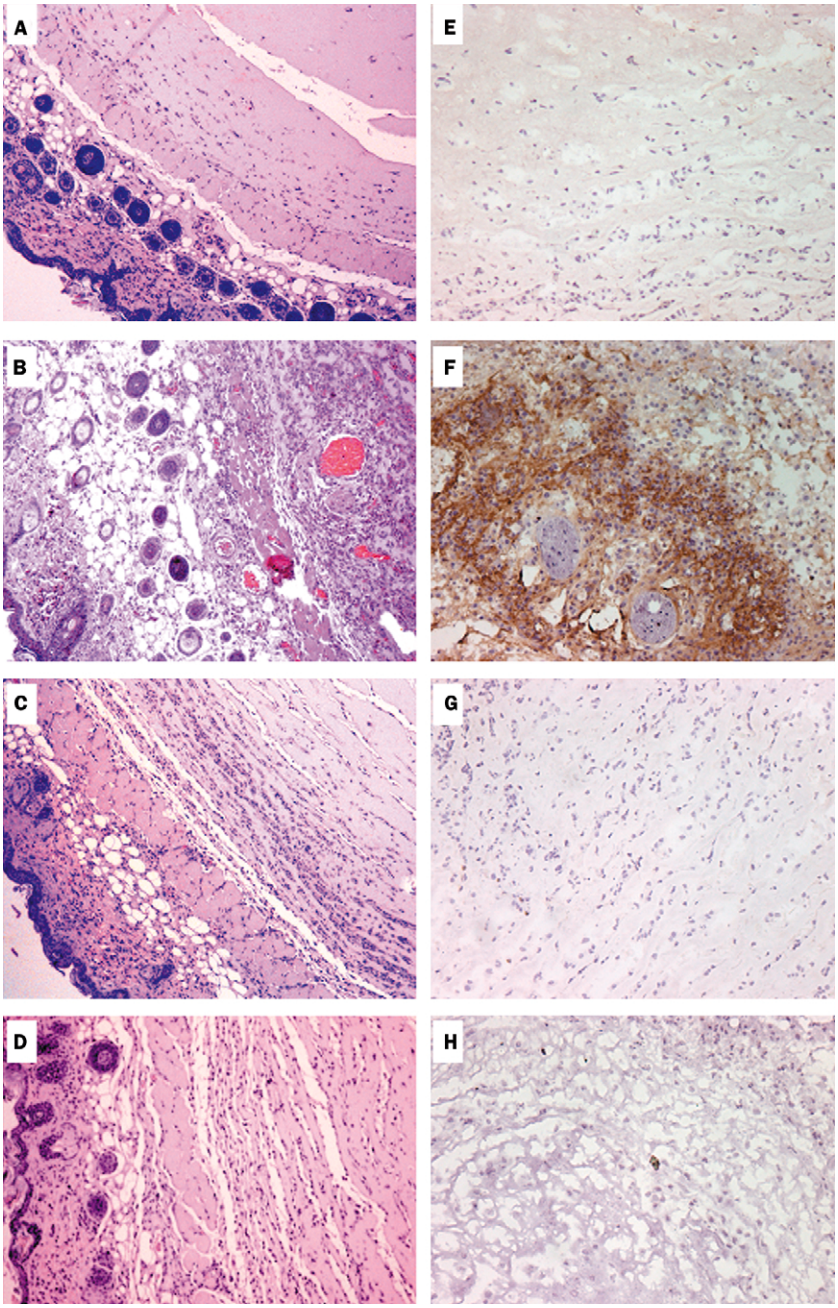

Figure 6. Indinavir or saquinavir block the formation of bFGF-induced angiogenic lesions in nude mice. Histological features (a–d) and FVIII expression (e–g) of lesions developed at the injection sites of mice injected with buffer (a and e) or bFGF and treated with saline (b and f), indinavir (c and g) or saquinavir (d and h).

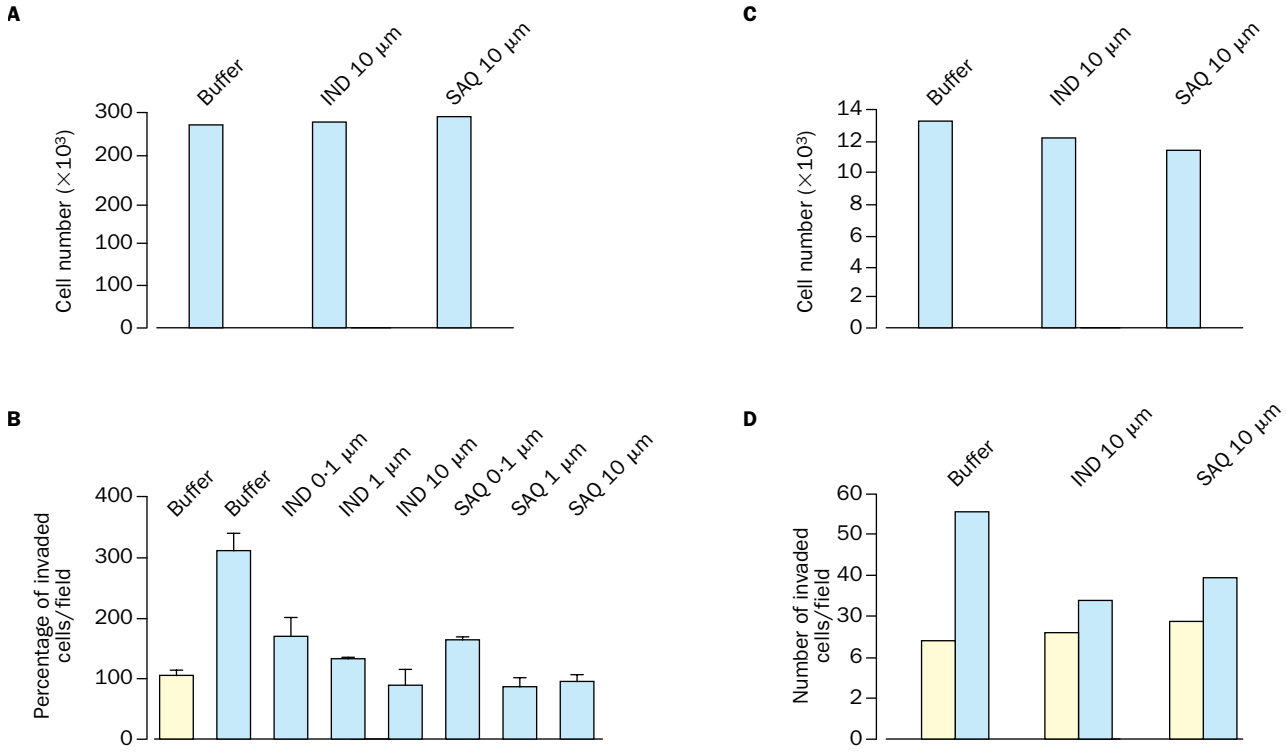

Figure 7. Protease inhibitors block the invasion but not the growth of human microvascular endothelial cells and aortic smooth muscle cells. Cell growth assays done with endothelial cells (a) or smooth muscle cells (c) in the presence of protease inhibitor or buffer. Cell invasion assays done in endothelial cells (b) or smooth muscle cells (d) by the Boyden chamber method in response to bFGF or buffer in the presence of protease inhibitor or protease-inhibitor-resuspension buffer. IND, indinavir; SAQ, saquinavir.

and spindle-cell infiltration compared with mice treated with saline (figure 4).[29] Since protease inhibitors inhibited development of KS-like lesions when treatment began 2 days before KS-cell injection and when it was given at the time of cell inoculation (figure 5), it seems that indinavir and saquinavir can block development of KS and induce regression, as has been observed in treated patients.

Protease inhibitors are strongly antiangiogenic

The histological features found in treated mice indicated that lesion regression and inhibition of KS growth is due to protease-inhibitor blockade of new blood vessel formation. Since KS is induced by the angiogenic factors produced in human or mice lesions by KS cells,[35] we tested whether indinavir or saquinavir may directly inhibit angiogenesis promoted by subcutaneous inoculation of bFGF or bFGF plus VEGF in nude mice.[38,49,55] Protease-inhibitor treatment either blocked lesion formation or greatly reduced the size of bFGF-induced angioproliferative macroscopic lesions.[29] Microscopic examination of the sites of bFGF injection showed greatly reduced angiogenesis and spindle-cell growth in protease-inhibitor-treated mice compared with control animals treated with saline and, in cases where inhibition was near total, the mice were almost indistinguishable from the negative controls (mice injected with medium but without KS cells; figure 6). This was confirmed by immunohistochemical staining of lesions with endothelial markers such as CD31[29] and FVIII-RA (figure 5) and quantified by computer-assisted analysis, which showed a significant reduction of angiogenesis in protease inhibitor-treated mice compared with controls.[29]

Protease inhibitors also blocked the formation of angiogenic lesions induced by the inoculation of a combination of VEGF and bFGF in nude mice.[29] In addition, protease inhibitors blocked angiogenesis induced by both bFGF and VEGF individually in the chorioallantoic membrane—a well known in-vivo assay for measuring angiogenesis and testing the potency of antiangiogenic compounds. Specifically, at similar concentrations to those present in plasma from treated patients, both indinavir and saquinavir inhibited bFGF or VEGF-induced formation of new vessels.[29] This effect was comparable to that observed with taxol, a cytotoxic drug with both antitumour and antiangiogenic activity that is used to treat KS and other solid tumours.[29,77] These data indicate that both indinavir and saquinavir directly block angiogenesis induced in vivo by both bFGF or VEGF as effectively as known antitumour and antiangiogenic drugs. We believe this effect is largely responsible for the reduced incidence and regression of KS in patients treated with protease inhibitors.

Protease inhibitors block of cell invasion

Angiogenesis consists of a series of co-ordinated sequential steps that are induced by angiogenic factors and enable endothelial cells and accessory cells, such as pericytes and smooth muscle cells, to form new vessels. These processes include: degradation of the blood vessel basement membrane and extracellular matrix by MMPs; directional migration of

cells into the perivascular space (endothelial-cell invasion); and endothelial-cell proliferation and differentiation.[47,80,81] Similarly, tumour growth, invasion, and metastasis require tumour cells to be capable of proliferating and invading tissues when the extracellular matrix is destroyed. Stromal cells and inflammatory cells infiltrating tumours participate in these processes by secreting paracrine factors, MMPs, and other proteases that increase both tumour-cell growth and extracellular-matrix degradation.[80] Thus, cell invasion, migration, proliferation, and degradation of the basement membrane and/or extracellular matrix are required both for angiogenesis and tumour progression. To investigate each of these mechanisms, protease inhibitors were tested in appropriate assays.

At concentrations present in plasma of treated individuals, neither indinavir nor saquinavir substantially affected bFGF-promoted proliferation, basal growth, or survival of macrovasculature-derived endothelial cells but they did block basement-membrane invasion.[29] These drugs have been show to have the same effects on microvascular endothelial cells (figure 7) and smooth muscle cells (figure 7).

Experiments with human primary KS cells derived from different patients also showed that indinavir and saquinavir have a selective effect on cell invasion.[29] Thus, both the antiangiogenic and antitumour effects of these drugs seem to be mediated through disruption of cell invasion. Furthermore, since tumour growth requires both angiogenesis and tumour-cell invasion, these data indicate that protease inhibitors may simultaneously disrupt two key processes that lead to tumour development and progression.

Indinavir and saquinavir inhibit MMP2 activation

The invasion of both endothelial and tumour cells is mediated by the proteolytic effects of MMPs.[80] In particular, active MMP2 is produced in response to inflammatory cytokines and bFGF by endothelial cells, is constitutively activated in KS and other tumours, and has a key role in both angiogenesis, KS, and tumour-cell invasion by degrading the vessel basement membrane and the extracellular matrix.[38,60,80] MMP2 is released by cells as an inactive zymogen (72 kD latent MMP2), which is proteolytically activated to the 64/62 kD active form at the cell surface through a complex mechanism involving several other proteases.[80]

Functional assays to study the effects of indinavir and saquinavir on MMP2 activity in endothelial cells have been done. No direct inhibitory effects on recombinant activated MMP2 were observed; this observation is in agreement with the finding that MMP2 cleaves after a glycine, whereas the HIV protease is an aspartyl protease, and that MMP2 has no sequence homology with the HIV protease catalytic site (the target of protease inhibitors).[82] However, indinavir and saquinavir prevented the conversion of latent MMP2 to its active form without affecting the synthesis of the latent zymogen form.[29] This indicates that protease inhibitors can block the activation of proteases that are key for angiogenesis and for tumour-cell invasion.[80] Furthermore, these agents may act by inhibiting one or more steps leading to MMP2 activation.

Blockade of tumour-cell invasion and angiogenesis

bFGF and VEGF are key to the development and progression of KS and for most other tumours.[47] In fact, no tumour growth occurs in the absence of angiogenesis and blocking this process in established tumours induces necrosis and tumour regression.[47] Moreover, prevention of cell invasion or MMP2 activation inhibits angiogenesis, tumour growth, and invasion. These observations led to investigation of the effects of indinavir and saquinavir on a frank tumour model, obtained by inoculating nude mice with the EA-hy 926 cell line. This cell line is a human hybrid between endothelial cells and a lung adenocarcinoma cell line that retains most of the endothelial-cell markers and is used to study angiogenesis.[29] Indinavir and saquinavir both effectively reduced the number and the size of EA-hy 926 cell-induced tumours in pretreated animals and in mice that started the treatment at the time of cell inoculation.[29] In addition, as already shown for small muscle cells, endothelial cells, and KS cells, these agents selectively blocked invasion but not proliferation or survival of EA-hy 926 cells.[29]

Finally, our recent data indicate that protease inhibitors are effective at blocking tumour growth and tumour-associated angiogenesis in other xenograft tumour models, without causing severe toxic effects. This has been observed in nude mice treated daily by intragastric gavage with doses of indinavir or saquinavir similar to those administered to HIV-infected patients and in mice inoculated with cells from human lung, breast, or colon adenocarcinomas, or with human cell lines of haemopoietic-cell origin (Sgadari and colleagues, unpublished data). Inhibition of tumour growth in these xenograft models was also associated with inhibition of tumour angiogenesis and tumour-cell invasion, with no or little effects on tumour-cell survival or proliferation (Sgadari and colleagues, unpublished data). Furthermore, preliminary data indicate that indinavir and saquinavir can inhibit pulmonary metastatic growth in a model of murine melanoma (Sgadari and colleagues, unpublished data).

Possible mechanisms of inhibition of AIDS-related KS by HAART containing a protease inhibitor

Effect	Mechanism
Immune system reconstitution	Improvement of immune surveillance against tumours
Reduction of HIV-1 Tat protein released by infected cells	Reduction of Tat effects on angiogenesis, KS progression
Inhibition of inflammatory production cytokine production	Reduction of cytokine-induced angiogenic factor and HHV8 reactivation
Improvement of anti-HHV8 activity mediated by cytotoxic T lymphocytes and natural killer cells	Control of HHV8 infection
Direct effect of angiogenesis	Inhibition of endothelial-cell invasion and MMP activation
Direct effect on KS and tumours	Inhibition of invasion of KS cells and tumour cells in vitro; prevention of KS lesion and tumour growth in vivo

KS, Kaposi's sarcoma; MMP, matrix metalloprotease.

In summary, these findings show that protease inhibitors modulate general mechanisms required for cell survival, growth, and invasion of most tumours.

Conclusions

This review brings together data which show that protease inhibitors have several unpredicted effects on host cell targets and pathways, in addition to HIV suppression and immune-reconstitution. These agents are antiangiogenic and antitumourigenic due to actions on cell invasion, MMPs, and proteolytic activation.[29] However, these compounds also inhibit the activation of monocytes, lymphocytes, and endothelial cells thereby reducing inflammatory-cytokine production and expression of adhesion molecules. Protease inhibitors can also alter the proliferation of PBMCs and activated T cells in a concentration-dependant manner.[12,15–18,22] These anti-inflammatory activities are likely to contribute to tumour control and might underlie therapeutic effects in patients with cancer. In addition to anti-inflammatory actions, protease inhibitors can block or modulate the activity of the cell proteasome.[12,15–18,22] Studies published in 2002 have shown that ritonavir and saquinavir promote in-vitro apoptosis of several tumour cell lines—including prostate cancer, glioblastoma, thymoma, and leukaemia cells—in association with altered protein degradation and sensitisation to radiotherapy.[14,83] Furthermore, ritonavir can inhibit development of KS lesions in mice inoculated with an immortalised KS cell line through effects on the cell proteasome and apoptosis.[15] However, the two distinctive actions of proteases—effects on MMPs and cell invasion, and modulation of the cell proteasome—are most likely to be controlled through different pathways targeted by particular protease inhibitors, combinations of agents, or concentrations. In fact, only ritonavir acts as a modulator of isolated proteasomes. In living cells, proteasome inhibition by indinavir, saquinavir, or ritonavir requires drug concentrations that are similar to or above the highest concentrations reached in the serum of treated patients.[5,12] In addition, a recent study has indicated that proteasome modulation at low drug concentrations is effective only when protease inhibitors are used in combination with non-nucleoside-analogue reverse-transcriptase inhibitors.[21] Finally, the block of cell invasion and MMP2 activation by indinavir or saquinavir occurs at drug concentrations that are 50 to 500 times lower than that required to elicit modulatory or inhibitory effects on the cell proteasome, cell survival, or cell growth.[12,17,20,21,29]

These data indicate that protease inhibitors should be investigated and exploited as novel antitumour drugs. The rationale behind many antitumour therapies curently in development is their ability to block endothelial-cell invasion and inhibit the cell proteasome;[13,47,80] however, protease inhibitors also directly inhibit KS and tumour-cell invasion so they exert additional effects on patients with KS and other tumours.

The effects of protease inhibitors reported in this review indicate that these drugs may be highly beneficial in specific categories of HIV-infected patients such as those with KS or those at high risk of developing KS. In view of the current trend to substitute protease inhibitors with other antiretroviral agents in HAART, this information has important implications.[84,85] Furthermore, since protease inhibitors seem to be promising antiangiogenic and antitumour compounds, they should be investigated for the treatment of other tumours in HIV-infected individuals and for KS in individuals who do not have HIV.

Protease inhibitors do not affect endothelial-cell growth or survival even at high concentrations, but they do block invasion by endothelial cells and tumour cells at the lowest drug concentrations detected in plasma of treated patients. This observation adds to evidence that these drugs have a favourable therapeutic index and lower toxicity than standard chemotherapeutics used in late-stage KS and that they should be the first therapeutic intervention in patients with newly diagnosed AIDS-related KS.[86] In addition, combined therapies with protease inhibitors should be investigated for the treatment of late nodular KS and other tumours that are often refractory to chemotherapy alone; evidence for this option comes from the remission of late AIDS-related KS resulting from treatment with protease inhibitors plus taxol.[87] However, conclusive evidence that protease inhibitors can be successfully used as antiangiogenic and antitumour drugs requires controlled clinical trials. An Italian multicentre clinical trial for the use of indinavir to treat KS in patients who are HIV-negative is about to start. This trial is an ideal setting for elucidating the anti-HIV and antitumour effects of these drugs and to validate the use of protease inhibitors in cancer therapy independently of HIV status.

The exact mechanism by which protease inhibitors act on MMP2 have not yet been clarified. They might inhibit one or more steps leading to MMP2 activation; however, other MMPs and molecules involved in angiogenesis and cell invasion may also be targets for protease inhibitors. This notion is consistent with the observation of several unpredicted effects, such as lipodystrophy syndrome experienced by patients treated daily for long periods.[1,5,82,88]

Many of intracellular targets of protease inhibitors are known pathogenetic factors, not only in KS or cancer but in several other diseases. In particular, chronic inflammation, and diseases that involve autoimmunity or angiogenesis diseases are associated with uncontrolled cell activation, inflammatory-cell infiltration, and increased production of inflammatory cytokines, angiogenic cytokines, MMPs, and other proteases.[89] Thus, more effective protease inhibitors and their analogues or derivatives may represent a future therapeutic tool for several immunological and reactive disorders.

The multiple effects of protease inhibitors raise the question of how these molecules can have such a wide range of

Search strategy and selection criteria

Published data for this review were selected from papers previously published by the authors on the topic or were identified by searches of MEDLINE. MeSH terms used were: "HIV protease inhibitors", "Kaposi's sarcoma", "regression", "incidence", "HHV8", "immune system", "metalloproteases". Additional references were selected from relevant articles. Only papers in English were included. Updates on the current trends for the treatment of HIV infection were found on the website of the HIV/AIDS Treatment Information Service at: http://www.hivatis.org.

effects on diverse cell targets. Indeed, many of these targets, previously thought to act via independent mechanisms, are most likely to be related. Several important findings indicate that MMP expression and activation is modulated by proteasome activity,[90] that MMPs are involved in cell apoptosis[80] and antigen-processing,[91] and that inhibition of the cell proteasome blocks cell activation via inhibiton of the NFκB pathway.[13] We are hopeful that the study of protease inhibitors' diverse effects will reveal an unexpected connection between the cellular pathways that these drugs have been shown to affect. At the same time, these investigations should lead to a better understanding of these pathways and the mechanisms of action of these antiretroviral agents.

Conflict of interest

None declared.

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Clinical picture

Bilateral bone metastasis in endometrial adenocarcinoma

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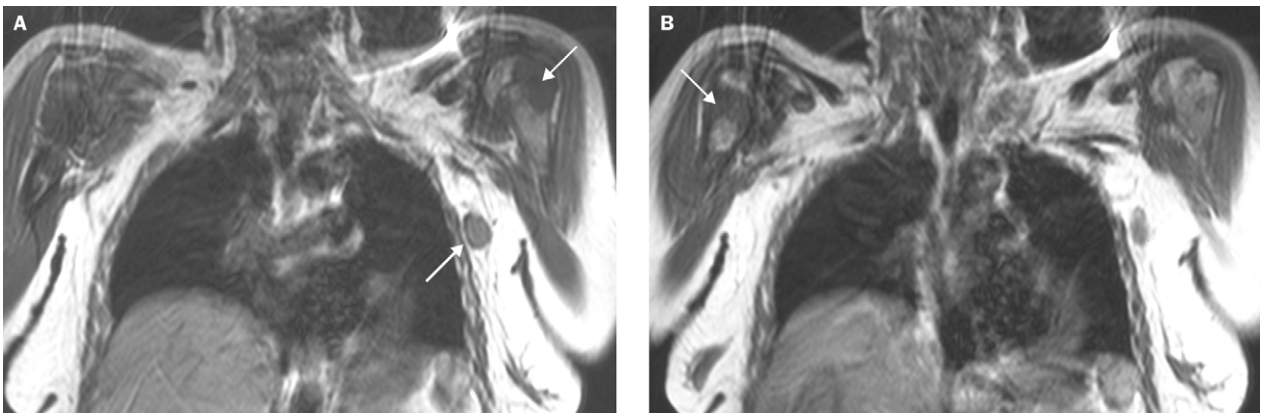

The occurrence of bone metastasis secondary to endometrial cancer is very rare. However, in such cases the primary neoplasm is often poorly differentiated, of a high stage, and indicative of recurrent disease. Endometrial metastases to the bone are generally restricted to the pelvis and vertebrae; peripheral skeletal metastases are very unusual and thought to result from the haematological spread of tumour cells.

Here, we present the case of a 51-year-old woman in whom postmenopausal bleeding was initially diagnosed as endometrial adenocarcinoma. A preoperative CT showed multiple enlarged retroperitoneal lymph nodes and tumour-like features on the omentum. Surgical staging included peritoneal washing, total hysterectomy, bilateral salpingo-oophorectomy, infracolic omentectomy, and pelvic and para-aortic lymph-node dissection. The final diagnosis was FIGO stage IIIc, grade 3 endometrioid adenocarcinoma. After surgery, the patient underwent radiotherapy but was readmitted to hospital 1 month later with shoulder pain in both sides, which was diagnosed as cervical discopathy. An x-ray examination showed non-specific radiolucent lesions suggestive of metastases around the head and neck regions of both humeri. MRI revealed metastatic lesions in the lateral portion of the head of the patient's left humerus (figure a) and the proximal metaphysis of the patient's right humerus (figure b). A soft-tissue mass suggestive of lymphadenopathy in the left axillary region adjacent to the thoracic cage was also seen (figure a). The patient was treated with palliative radiotherapy to relieve the pain. 6 months after treatment, she was alive, but additional scintigraphic bone scans showed multiple metastatic bone lesions in the vertebral colon.

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