ANTITUMOUR EFFECTS OF ANTIRETROVIRAL THERAPY

Paolo Monini, Cecilia Sgadari, Elena Toschi, Giovanni Barillari and Barbara Ensoli

Abstract | Infection by human immunodeficiency virus (HIV) is associated with an increased risk of certain tumours, particularly Kaposi's sarcoma, non-Hodgkin's lymphomas and cervical cancer. However, the incidence of these tumours in HIV-infected patients has decreased significantly since the widespread use of highly active antiretroviral therapy (HAART). This effect cannot be solely explained by the ability of these drugs to suppress HIV replication and thereby reconstitute the immune system. Recent studies have shown that inhibitors of the HIV aspartyl protease, which are widely used in HAART, have direct anti-angiogenic and antitumour effects that are unrelated to their antiviral activity. So these drugs might be used to treat cancer in patients who are not infected with HIV.

NAIVE AND MEMORY T-CELL REPERTOIRE
Mature T cells that have not yet encountered antigens are called naive T cells. Following antigen recognition, naive T cells become activated, proliferate and some differentiate into memory T cells. Both the naive and memory T-cell repertoires are progressively shrunk in HIV-infected patients.

AIDS Division, Department of Infectious, Parasitic and Immune Mediated Diseases, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy.
Correspondence to B.E.
e-mail: Ensoli@iss.it
doi:10.1038/nrc1479

Until a few years ago, infection by the human immunodeficiency virus (HIV) was inexorably leading to AIDS, characterized by immunodeficiency, opportunistic infections and increased incidence and aggressiveness of certain types of tumour, particularly Kaposi's sarcoma, B-cell lymphomas and anogenital cancer (TABLE 1)[1]. The advent of the highly active antiretroviral therapy (HAART), however, has turned HIV infection into a manageable disease[2]. HAART is a combination therapy against HIV that comprises several active molecules that block multiple viral targets (BOX 1). At present, HAART uses drugs directed against the viral reverse transcriptase — nucleoside and non-nucleoside reverse-transcriptase inhibitors (NRTIs and NNRTIs, respectively) — and drugs directed against the viral aspartyl protease, known as HIV-protease inhibitors (HIV-PIs)[3]. NRTIs act by blocking the HIV reverse-transcriptase active site, whereas NNRTIs inhibit the activity of HIV reverse transcriptase by binding to other sites present on the enzyme (allosteric inhibition)[3]. HIV-PIs are peptide mimetic drugs that have been designed to mimic the phenylalanine–proline peptide bond, which is targeted by the viral protease, but is not cleaved by any of the known mammalian endopeptidases. This confers a remarkable specificity of action to HIV-PIs that, on short-term treatment, show only mild side effects and a tolerable toxicity. So, two types of HAART regimens are in use at present, and include a combination of traditional NRTIs with either NNRTIs (NNRTI–HAART) or HIV-PIs (PI–HAART) — these are similarly effective in suppressing HIV infection[3].

The rationale for these combination therapies is to inhibit several steps of the viral life cycle. Combination regimens based on HIV-PIs or NNRTIs have been shown to be more effective than single or dual combinations of NRTIs in suppressing HIV replication, and in preserving or reconstituting both NAIVE AND MEMORY T-CELL REPERTOIRES, which delays or reverts the onset of AIDS[4–7]. HAART has also led to a lower incidence and/or regression of Kaposi's sarcoma, non-Hodgkin's lymphomas (NHLs) and intra-epithelial anogenital tumours[8,9].

Antiviral drugs included in HAART regimens are known to exert a direct antitumour action. In particular, azidothymidine (AZT), one of the most widely used NRTIs, has been shown to block telomerase activity, to induce TRIAL-mediated apoptosis of lymphomatous cells and to reverses cisplatin resistance in patients with cancer[10–12]. AZT therapy also leads to a complete remission of advanced Kaposi's sarcoma in HIV-infected patients treated with chemotherapy[13]. Therapies based on single or dual NRTI regimens have also decreased the incidence of Kaposi's sarcoma and NHLs[14–16]. However, this reduction is much lower than the marked decrease in the incidence of these tumours in patients treated with HAART.

Summary

- Infection with the human immunodeficincy virus (HIV) increases a patient's risk for developing certain types of cancers.
- Immune hyperactivation, due to uncontrolled HIV replication, and immune deficiency have been shown to be the key factors in the initiation and progression of these cancers, particularly Kaposi's sarcoma and B-cell lymphomas, in HIV-infected patients.
- Highly active antiretroviral therapy (HAART) reduces cancer risk and tumour burden in HIV-infected individuals.
- The effects of these drugs cannot be entirely explained by their ability to suppress HIV replication and restore normal immune function — tumour development is not always correlated with a patient's viral load or level of immune reconstitution.
- Direct antitumour effects of HAART could be related to specific actions of the protease inhibitors included in this therapeutic cocktail, such as ritonavir, saquinavir, indinavir and nelfinavir. These drugs have been shown to inhibit proliferation and induce apoptosis in cultured cancer cells, to block endothelial- and tumour-cell invasion, *in vivo* angiogenesis and tumour growth, as well as the inflammatory response.
- The ability of these drugs to prevent tumour growth and progression might be mediated by their ability to inhibit proteasome function and the activity of matrix metalloproteinases.
- As HAART has already been shown to be safe and effective for the treatment of patients with AIDS, these drugs might be exploited, alone or in combination with conventional cytotoxic therapy, for the treatment of non-HIV-infected patients with cancer.

These antitumour effects of HAART regimens have been attributed to the extraordinary efficacy of this drug combination in suppressing HIV infection, and to the consequent reconstitution of cellular immunity against viruses implicated in AIDS-associated malignancies, including Epstein–Barr virus (EBV), Kaposi's-sarcoma-associated herpesvirus (KSHV) or human papillomavirus (HPV). This model, however, does not explain why these antiretroviral regimens did not show similar effects on other HIV-related proliferative diseases associated with the same viruses, including EPIDERMODYSPLASIA VERRUCIFORMS, MULTICENTRIC CASTLEMAN'S DISEASE, HODGKIN'S DISEASE or invasive cervical cancer[8,17,18]. Moreover, some HIV-infected patients who still experience persistent and profound immune deficiency despite HAART treatment still have a much lower risk of developing NHLs, compared with similarly immune-suppressed individuals who have been treated with single or dual combinations of NRTIs[19]. This indicates that HAART could have direct antitumour effects that are unrelated to the use of NRTIs and are independent of immune reconstitution.

There is much evidence that HIV-PIs have direct effects on angiogenesis, tumour growth and tumour

Table 1 | **HIV-associated malignancies**

Tumour type	Relative risk*	Viral co-factors (prevalence of viral DNA in tumours)	Reported effects of HAART‡ on incidence	Reported effects of HAART‡ on outcome
AIDS-defining§				
KS	258	HHV8 (100%)	Decreased	Regression/remission
NHL	78.1	EBV, HHV8	Decreased	Improved survival/ regression
Burkitt's (classic form)	103	EBV (30%)	Unchanged/decreased	Improved survival
DLCL, centroblastic	NA	EBV (40%)	Unchanged/decreased	Improved survival
DLCL, immunoblastic	134	EBV LMP1 (90%)	Decreased	Improved survival
PCNS	175	EBV LMP1 (100%)	Decreased	Regression (anecdotal evidence)
PEL	NA	HHV8 (100%), EBV (80%)	NA	Regression (anecdotal evidence)
Uterine cervix (invasive)	8.8	HPV (100%)	Unchanged	Regression (anecdotal evidence)
Non-AIDS-defining§				
HD	11	EBV LMP1 (80–100%)	Unchanged/increased	Improved survival
Lung	2.8	?	Increased	Prolonged time from HIV infection to tumour development
Liver	5.1‖	HBV; HCV	Unchanged	Worsening
Skin (non-KS)	20.9‖	HPV (non-melanomatous)	?	?
Anal	49.9	HPV	?	Regression (anecdotal)
Uterine cervix (pre-invasive)	9.3	HPV	?	No effect/regression; longer time to relapse
Testis	1.4	?	?	Unchanged survival

*Calculated as standardized incidence ratio (SIR) in the United States population before the widespread use of highly active antiretroviral therapy (HAART). SIRs are from REF. 26 for AIDS-defining tumours, Hodgkin's disease (HD), lung cancer, anal cancer, pre-invasive cervical cancer and testicular cancer; and from REF. 189 for liver and skin cancer. ‡For references relative to AIDS-defining tumours as well as to HD, pre-invasive cervical cancer and anal cancer, see main text; for other non-AIDS-defining tumours, see REFS 8,190,192,193. §AIDS-defining tumours, like opportunistic infections, are considered to mark AIDS onset in HIV-infected individuals. ‖SIRs were calculated for men. DLCL, diffuse large-cell lymphoma; EBV, Epstein–Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HHV8, human herpesvirus type 8; HPV, human papillomavirus; KS, Kaposi's sarcoma; LMP1, latency membrane protein 1; NA, not available; NHL, non-Hodgkin's lymphoma; PCNS, primary-nervous-system lymphoma; PEL, primary effusion lymphoma.

EPIDERMODYSPLASIA VERRUCIFORMIS
A genetic disease characterized by increased susceptibility to human papillomaviruses.

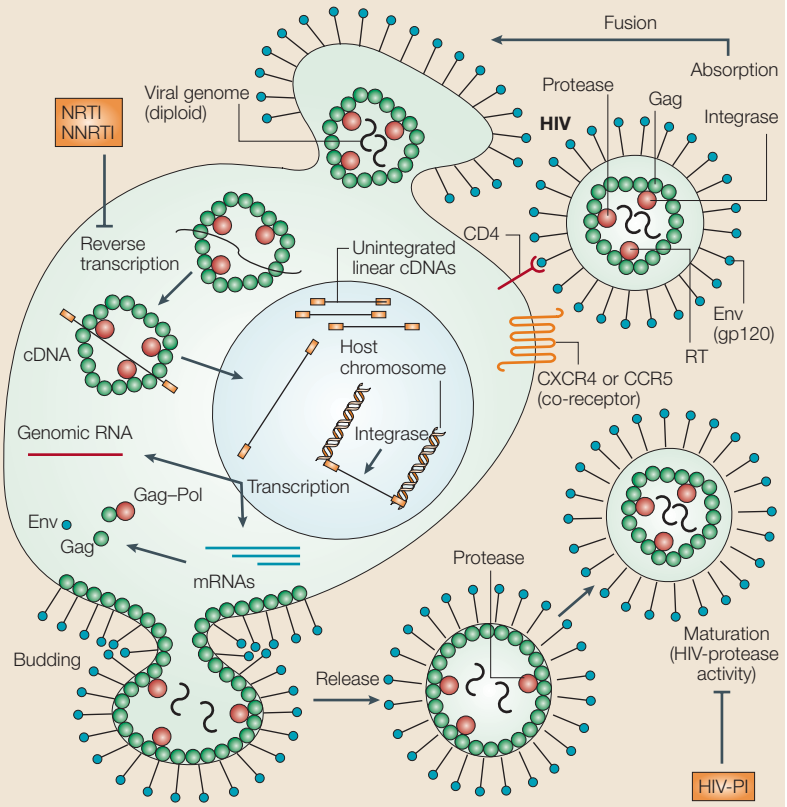
MULTICENTRIC CASTLEMAN'S DISEASE
Lymphoproliferative disorder characterized by reactive lymph nodes with expanded germinal centres and B-cell proliferation. In HIV-infected individuals, the plasma-cell variant is the predominant form, which, in this setting, is invariably infected by KSHV.

HODGKIN'S DISEASE
A heterogeneous lymphoma entity originating from germinal-centre or post-germinal-centre B cells. The classical form has a post-germinal-centre phenotype and is the predominant form in HIV-infected individuals.

immunity that are unrelated to their antiviral effects. These seem to be mediated through specific activities on the proteasome and on MATRIX METALLOPROTEINASES (MMPs)[20–22].

Box 1 | **HIV life cycle**

Human immunodeficiency virus (HIV) infects several cell types, including CD4⁺ T cells, monocytes and dendritic cells. The infectious cycle begins with the adsorption of viral particles to the receptor CD4, which is present at the cell surface. This interaction, which is mediated by the HIV envelope (Env) protein gp120, leads to subsequent interaction of the gp120 V3 loop region with a co-receptor, usually a member of the seven-membrane-spanning chemokine-receptor families, the most important being CCR4 or CXCR5 (REF. 187). After virus adsorption, the viral and cell membranes fuse together, and the viral 'core' (which includes the diploid viral genome) is released into the cytoplasm, where the virion-associated reverse transcriptase is activated and begins synthesizing viral cDNA. This is subsequently transported to the cell nucleus, where another virion-associated enzyme, the HIV integrase, catalyses the insertion (integration) of the viral cDNA into the host-cell genome. Transcription of the integrated viral cDNA leads to the production of genomic (unspliced) and messanger (spliced) RNA (mRNA) molecules that are transported to the cell cytoplasm. Translation of HIV mRNAs leads to the production of Env proteins and immature precursors of capsid (Gag) and viral polymerase (Pol) proteins. Immature Gag and fused Gag–Pol precursors are transported to the cell membrane, where viral progeny begin assembling and 'bud' from the infected cells. Viral particles released following budding, however, do not contain the characteristic HIV condensed core and are not infectious. Virus infectivity is acquired after particle maturation, which is mediated by the virion-associated HIV aspartyl protease. This enzyme cleaves the immature Gag and Gag–Pol precursors into functional polypeptides. These crucial steps of the HIV life cycle are targeted with nucleoside and non-nucleoside reverse-transcriptase (RT) inhibitors (NRTIs and NNRTIs, respectively), and with HIV-protease inhibitors (HIV-PIs).



HIV infection and tumour development

AIDS-associated malignancies are all associated with DNA tumour viruses such as EBV, KSHV and HPV (TABLE 1). For this reason, these malignancies are commonly considered to be the result of diminished immune surveillance against viruses and virus-infected tumour cells. The beneficial effects of HAART on these tumours have therefore been interpreted as the result of drug-mediated HIV suppression and immune reconstitution. This is supported by several findings. For example, Kaposi's sarcoma is a multifocal angiogenic tumour characterized by proliferating spindle-shaped cells of endothelial-cell origin that are latently infected by KSHV[23]. Studies indicate that KSHV load and antibody titres, as well as the patient's number of CD4⁺ HELPER T LYMPHOCYTES — the main targets of HIV replication[24–26,32] — are independent determinants for risk of developing Kaposi's sarcoma[25–31]. Similarly, EBV load is increased in patients before development of B-cell lymphoma, whereas specific immune responses against the virus are decreased[33–35]. The relative risk of AIDS-associated malignancies increases progressively as a function of the progressive decline of CD4⁺ T-cell counts[27].

Nevertheless, the relation between immune deficiency and tumour development is not straightforward. In fact, only certain types of AIDS-associated tumours arise in immunodeficient patients. In particular, NHL subtypes including IMMUNOBLASTIC LYMPHOMAS and PRIMARY-NERVOUS-SYSTEM LYMPHOMAS, along with Burkitt's-like lymphomas, typically develop in patients with very low CD4⁺ T-cell counts. On the other hand, the incidence of other NHL subtypes such as CENTROBLASTIC DIFFUSE LARGE-CELL LYMPHOMAS, along with classic BURKITT'S LYMPHOMA, Hodgkin's disease, cervical cancer and, most notably, Kaposi's sarcoma, increases in patients who have significantly higher CD4⁺ T-cell numbers[1,19,36–41].

The overall risk of tumour development is very high in HIV-infected individuals (TABLE 1), but the relative increase in tumour risk with stepwise decreases in CD4⁺ T-cell counts is only marginal[27]. So the risk of tumour development increases steeply as CD4⁺ T-cell counts decline below a certain threshold. Once below this threshold, cancer risk becomes less dependent on further CD4⁺ T-cell loss[27]. However, evidence indicates that this hypothetical CD4⁺ T-cell count threshold can be very high in certain individuals. In particular, in HIV-infected homosexual men, the incidence rate of Kaposi's sarcoma increases by more than 1000-fold before a consistent CD4⁺ T-cell decline[1]. So, CD4⁺ T-cell loss and consequent immune deficiency cannot fully explain the increased incidence of certain malignancies in HIV-infected individuals. Indeed, several recent studies show that immune activation causes and precedes the development of immune deficiency in HIV infection[42–45]. Sustained and uncontrolled HIV replication leads to continuous antigenic stimulation and to chronic T-cell activation and proliferation, which, in turn, generates a continuous drain of naive and memory T cells that become activated, proliferate, die by apoptosis or re-enter the pool of memory T cells.

However, this exhausts the pool of naive T cells, impairing the capacity to mount antigen-specific immune responses[42–45] (BOX 2).

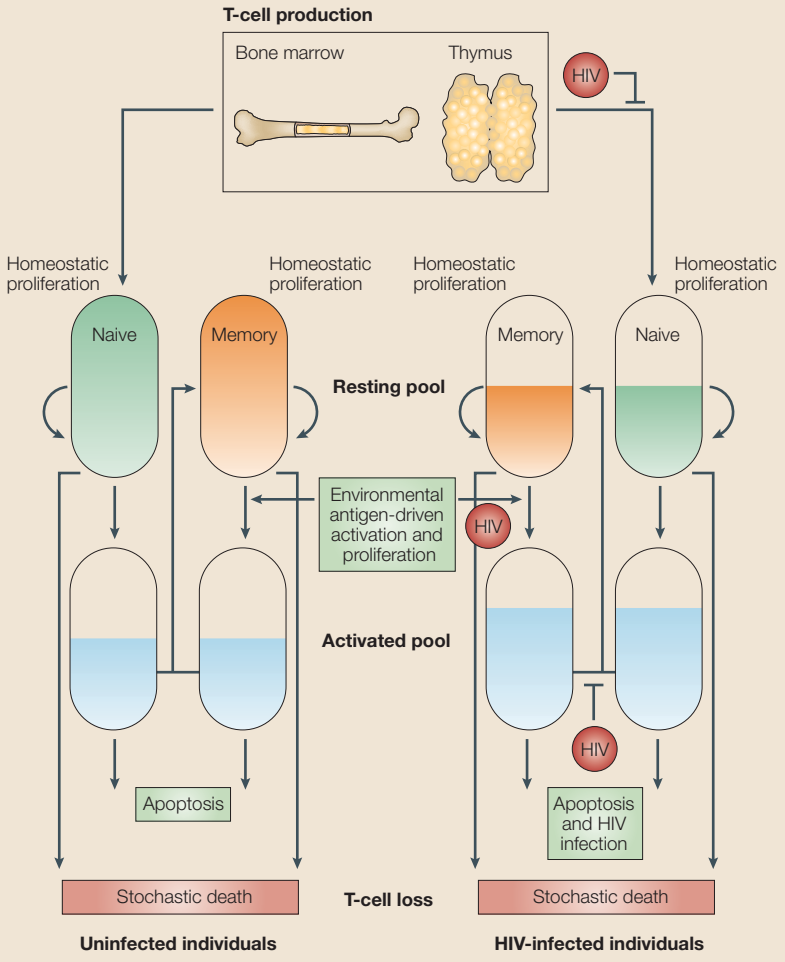
Several other studies also indicate that immune activation, rather than immune deficiency, is the key factor in the initiation of Kaposi's sarcoma and B-cell lymphomas. In particular, Kaposi's sarcoma begins as a reactive process, characterized by blood-vessel activation (expression by endothelial cells of adhesion molecules and $\alpha_v\beta_3$- and $\alpha_5\beta_1$-integrins), tissue infiltration by activated $CD4^+$ and $CD8^+$ T cells, production of angiogenic factors, increased expression of MMPs, and intense and aberrant angiogenesis[23]. Extracellular HIV-1 Tat PROTEIN, released by HIV-infected cells, binds the $\alpha_v\beta_3$- and $\alpha_5\beta_1$-integrins, increasing the effects of the angiogenic factors[46–53]. These processes can precede the appearance of spindle-shaped cells of endothelial or monocytic cell origin (the so-called Kaposi's-sarcoma spindle cells), which are believed to be the tumour cells of Kaposi's sarcoma[54,55] and becomes progressively infected by KSHV[23].

Evidence indicates that all these events, including KSHV reactivation and virus dissemination to tissues, are driven by the actions of T_H1-TYPE PRO-INFLAMMATORY CYTOKINES, particularly interferon-γ (IFNγ), interleukin-1β (IL-1β) and tumour-necrosis factor-α (TNFα), which are increased in patients infected by HIV[23,46,48,55–60]. This is also supported by the observation that treatment of HIV-infected patients with IL-2, TNFα, or IFNγ resulted in onset or progression of Kaposi's sarcoma[61,62]. Furthermore, a CD8/T_H1-type immune-activation profile is a specific trait of individuals who are at risk of forms of Kaposi's sarcoma that are not associated with HIV infection, including CLASSIC KAPOSI'S SARCOMA[23,63–66] and AFRICAN KAPOSI'S SARCOMA[23,67–69]. Moreover, T_H1-type cytokines are also likely to be involved in the initiation of POST-TRANSPLANT KAPOSI'S SARCOMA. In fact, only a small fraction of KSHV-infected organ recipients develop Kaposi's sarcoma following initiation of immune-suppressive therapy[70], whereas disease onset is most frequently preceded by acute or chronic graft rejection or opportunistic infections, which induce strong T_H1-type responses[71,72]. AIDS-associated B-cell lymphomas are also preceded by chronic antigen-dependent B-cell stimulation leading to a persistent and generalized lymphadenopathy that, in turn, promotes the clonal expansion of pre-neoplastic antigen-specific B-cell populations[73,74]. Furthermore, an increased EBV load precedes the development of B-cell lymphoma[34], whereas extracellular Tat increases B-cell proliferation and induces B-cell lymphomas in mice[75,76].

So, AIDS-associated malignancies are multifactorial and their progression is promoted by the combined effects of immune activation, viral cofactors (including tumour viruses and extracellular Tat) and immune deficiency. In this regard, two variables are most often measured to monitor HIV infection and the efficacy of antiretroviral therapies — the number of $CD4^+$ T cells, which is a measure of immune integrity or deficiency, and HIV load. In HAART-treated patients, HIV load remains high in non-responders or in patients showing persistent viraemia despite an increase in their $CD4^+$ T-cell numbers. These individuals have high or intermediate levels of immune activation compared with patients who respond to HAART. So, HIV load might be regarded as an indirect measure of immune activation in treated patients. Recent data, however, have indicated that the effects of HAART on AIDS-associated tumours are not always well correlated to either one of these two key variables, so HAART might exert direct antitumour

Box 2 | HIV-induced immune activation leads to immune deficiency

In a normal immune system (left side of figure), exposure to environmental antigens continuously activates naive and/or memory T cells, driving their replication and establishing and maintaining a pool of proliferating cells (activated pool). Activated cells become effectors and undergo apoptosis or survive to replenish the 'resting pool' as memory cells. Naive and memory T cells also die by 'natural' (stochastic) events — this is compensated by homeostatic proliferation. Production and maturation of T cells in the bone marrow and thymus also compensate for these losses. In patients infected with human immunodeficiency virus (HIV), however, several mechanisms perturb T-cell homeostasis (right side of figure). In the immune system of an individual infected with the HIV, the 'activated pool' of T cells is targeted by the virus, and this impairs the replenishment of the memory compartment. The input of T cells from the bone marrow and thymus is also reduced due to direct and indirect effects of HIV infection[188]. But the main force that drives the immune system into collapse and that leads to overt immune deficiency in HIV infections is the chronic antigenic stimulation that results from uncontrolled HIV replication[42]. As a result, the activated cell pool expands, whereas the pools of naive and the memory T cells progressively shrink.



actions that act independently of immune reconstitution or suppression of immune activation.

HAART inhibits AIDS-associated tumours

Following HAART initiation, patients show a sudden decrease in HIV replication associated with a reduction of both T-cell and B-cell activation and turnover rates[4,43,44,77–81]. This leads to an increase in memory and naive CD4$^+$ and CD8$^+$ T-cell counts, normalization of the B-cell compartment and, eventually, sustained immune reconstitution[4,78,80,82,83]. These effects of HAART are associated with a significant decrease in the incidence of AIDS-associated malignancies — particularly in the incidence of Kaposi's sarcoma and NHLs — as well as with tumour regression, prolonged time to failure of tumour treatment and longer survival times[8,84–93]. These positive outcomes have been generally attributed to restoration of the immunological control of viruses, including EBV, KSHV and HPV[19,84,94–97].

Studies comparing the incidence of lymphomas before or after the advent of HAART indicated that HAART resulted in a significant decrease in the incidence of systemic immunoblastic lymphomas and primary-nervous-system lymphomas, but not of the other lymphoma subtypes[8,15,98,99]. This has been generally attributed to drug-mediated reconstitution of immune responses against cells that express immunogenic EBV latency gene products, including the latency membrane protein 1 (LMP1) oncoprotein, which is prominently expressed by immunoblastic lymphomas and primary-nervous-system lymphomas (TABLE 1)[74,100–102]. However, recent retrospective studies that compared the incidence of lymphomas in groups of patients treated with HAART or with NRTIs, and patients who did not receive antiretroviral therapy showed that the incidence of all lymphoma subtypes is reduced in patients who have received HAART. So, HAART reduces the incidence of NHLs whether or not their pathogenesis depends on immune suppression[19,95].

A high rate of tumour regression and/or complete remission is frequently observed in patients with Kaposi's sarcoma and NHL who have been treated with either chemotherapy or radiotherapy and HAART, or with HAART alone[9,84,88,90,91,96,103–117]. Although larger, controlled studies are required, these data indicate that HAART not only reduces tumour incidence in HIV-infected individuals, but it also induces an antitumour response. However, although increased CD4$^+$ T-cell counts and decreased or undetectable levels of HIV and KSHV viraemia are independent predictors of the response of Kaposi's sarcoma to HAART[84,103,105,108,118], several studies reported a lack of correlation between one or more of these determinants and tumour regression[107,119–121]. A recent study has shown that HAART prolongs the length of time that patients with Kaposi's sarcoma respond to chemotherapy, but this effect is not well correlated with restoration of CD4$^+$ T cells or with control of HIV viraemia[87]. In another recent study, reconstitution of immune responses against KSHV and decreases of plasma viraemic levels following HAART have been shown to require several months of therapy (over 24 months) — much longer than the rapid response of Kaposi's sarcoma to these drugs[122]. This tumour, in fact, typically responds to HAART within the first weeks of treatment, often resulting in complete disease remission after only a few months of therapy[122].

Similarly, regression of cervical intraepithelial neoplasia occurs following HAART initiation despite persistence of HPV DNA in the cervix[9,117]. By contrast, in untreated patients, persistence of HPV infection leads to lesion progression[123,124]. Although regression of cervical intraepithelial neoplasia during HAART is associated with immune reconstitution, a lack of correlation between CD4$^+$ T-cell counts, HIV load, clearance of HPV infection and lesion regression has been reported[9,96,117]. All these data confirm that regression or resolution of Kaposi's sarcoma, NHLs and cervical intraepithelial neoplasias in response to HAART are mediated, at least in part, by effects that are not due to suppression of HIV infection, inhibition of antigen-driven immune activation, or HAART-mediated immune reconstitution.

Antitumour effects of HIV-protease inhibitors

The effects of HAART on tumour incidence and regression have been most frequently studied in cohorts or groups of patients treated with PI–HAART rather than NNRTI–HAART[9,84,103–109,111,112,114–116]. This reflects, at least in part, the fact that PI–HAART was in use before the advent of NNRTI–HAART. However, this might also indicate that the unexpected antitumour effects of HAART are related to specific actions of HIV-PIs. This was addressed by directly comparing the impact of PI–HAART and NNRTI–HAART on patients with Kaposi's sarcoma or NHLs. In three recent studies, both regimens were found to be equally effective in reducing the incidence of Kaposi's sarcoma or NHLs[14,16,125]. Contrasting results, however, have been obtained when looking at tumour regression or relapse. A recent study analysed the response of Kaposi's sarcomas in a series of ten patients treated with PI–HAART or NNRTI–HAART. Both regimens were found to induce partial regression of Kaposi's-sarcoma lesions with a similar frequency[126]. However, complete remission of Kaposi's sarcoma occurred more frequently in patients treated with PI–HAART than in those treated with NNRTI–HAART[126]. These patients had very low CD4$^+$ T-cell counts at the beginning of the study, and only small and partial CD4$^+$ T-cell recovery after therapy. So, in patients with low immune activity, HIV-PIs were more efficient at inducing complete response than NNRTI–HAART.

Two other small studies reported the effects of switching patients from PI–HAART to NNRTI–HAART after resolution of Kaposi's sarcoma. Although one study reported that switching these drugs had no effect on relapse, the other study reported that it was consistently associated with tumour relapse, even though both regimens were equally effective against HIV infection[127,128]. Although larger retrospective and prospective studies are required to draw a firm conclusion, these data indicate that the antitumour effects of HAART might, at least in part, be related to specific actions of HIV-PIs.

MATRIX METALLOPROTEINASES (MMPS)
Enzymes that degrade the extracellular matrix and are required for tumour-cell invasion of basement membranes and migration in interstitial tissue. MMPs are involved in angiogenesis, wound healing, tumour invasion, inflammation and all processes requiring tissue remodelling.

HELPER T CELLS
T lymphocytes that express the surface molecules CD3 and CD4, and recognize antigen peptides presented by MCH-class-II molecules. Following antigen recognition, these cells produce cytokines that activate B-cells, macrophages and granulocytes, and that induce differentiation of cytotoxic T lymphocytes into effector cells.

IMMUNOBLASTIC LYMPHOMA
A subtype of NHLs that belongs to the group of the diffused large-cell lymphomas, accounting for about 25% of HIV-associated lymphomas. It is associated with EBV infection and expression of LMP1.

PRIMARY-NERVOUS-SYSTEM LYMPHOMA
A NHL subtype, usually with immunoblastic features, that arises in the central nervous system. This subtype is invariably associated with EBV infection, and expresses the viral oncoprotein LMP1.

CENTROBLASTIC DIFFUSE LARGE-CELL LYMPHOMA
A systemic subtype of NHL belonging to the group of the diffused large-cell lymphomas, accounting for about 10% of HIV-associated lymphomas. About 30% of these lymphomas are infected with EBV but do not express the viral oncoprotein LMP1.

BURKITT'S LYMPHOMA
A systemic subtype of NHL that accounts for about 30% of HIV-associated lymphomas. It is characterized by c-MYC alteration due to chromosomal translocation, and by p53 inactivation. About 30% of cases are associated with EBV infection, and tumour cells do not express the viral oncoprotein LMP1.

Table 2 | **Direct effects of HIV-PIs on tumours**

HIV-PI	Effect	Experimental model	Drug dose/ concentration	Mechanism(s) of action	Molecular target(s)	References
Ritonavir	Inhibition of KS growth	KS cell line transplanted into immunodeficient mice	HIV therapeutic dose	Blocks KS-cell apoptosis endothelial-cell activation (through NF-kB inhibition)	Proteasome	130
Ritonavir	Inhibition of tumour-cell proliferation and induction of apoptosis	KS cell line and other human tumour cell lines; syngenic mouse tumour model	Similar to or above patient's peak plasma concentration (3–50 μM); HIV therapeutic dose	Accumulation of the cyclin-dependent kinase inhibitor WAF1	Proteasome	129,130
Saquinavir and indinavir	Necrosis of KS lesions	Primary (non-immortalized) KS cells transplanted into immunodeficient mice	HIV therapeutic dose	Blocks neoangiogenesis	MMPs, possibly integrins	22
Saquinavir and indinavir	Inhibition of growth and metastasis of tumours of various origin	Human or syngenic tumour grafts in immunodeficient mice	HIV therapeutic dose	Blocks angiogenesis, tumour-cell invasion, ECM remodelling	MMPs, possibly integrins	22,194
Saquinavir and indinavir	Inhibition of cell invasion (with no effects on cell viability/proliferation)	Human endothelial cells and tumour cell lines	Patient's steady-state plasma concentration (0.1–1 μM)	Blocks MMP2 proteolityc activation	MMP2, MT1-MMP, possibly $\alpha_v\beta_3$-integrin	22,194
Saquinavir	Tumour-cell apoptosis and radiosensitization	Human tumour cell lines	Above patient's peak plasma concentration (50–100 μM)	Inhibition of NF-κB function	Proteasome	21

The potential antitumour effect of other human immunodeficiency virus protease inhibitors (HIV-PIs), including nelfinavir, amprenavir, lopinavir and atazanavir, is under study. ECM, extracellular matrix; KS, Kaposi's sarcoma; MMP, matrix metalloproteinase.

Many studies have therefore been aimed at identifying non-virological actions of the most widely used HIV-PIs, including ritonavir, saquinavir, indinavir and nelfinavir. They have found that HIV-PIs directly affect several pathways involved in tumour-cell proliferation and survival, angiogenesis, invasion, inflammation, and antitumour immunity in HIV-free models (TABLES 2,3). For example, ritonavir and saquinavir inhibited the proliferation of tumour cell lines of lymphoblastoid origin, including lymphoma cells and myeloid leukaemia cells, fibrosarcoma and mastocytoma cells, as well as immortalized Kaposi's-sarcoma cell lines[129,130]. This effect was associated with the induction of apoptosis in tumour cells, but no effect on proliferation or survival was observed with non-tumour cells, including non-transformed immortalized fibroblasts or primary macrophages[129,130]. Furthermore, saquinavir induced apoptosis in prostate cancer cells, glioblastoma cells and lymphocytic leukaemia cells, and sensitized tumour cells to cell death by ionizing radiation[21] (TABLE 2).

HIV-PIs also inhibit angiogenesis and tumour growth by blocking cell invasion (TABLE 2). In particular, studies showed that indinavir and saquinavir inhibited the development of Kaposi's-sarcoma-like angioproliferative lesions induced in nude mice following injection of primary Kaposi's sarcoma cells derived from human lesions[22]. In treated mice, these drugs promoted the formation of a large central necrotic area and a marked reduction of neoformed vessels, oedema and spindle-cell infiltration[22]. These effects of HIV-PIs were found to be due to inhibition of basic fibroblast growth factor (bFGF)- and vascular endothelial growth factor (VEGF)-mediated angiogenesis; both proteins are produced by Kaposi's-sarcoma cells[22,50,58,59,131,132]. Indinavir and saquinavir have also been shown to inhibit the formation of angiogenic lesions directly induced by angiogenic factors in nude mice, as well as bFGF or VEGF-induced angiogenesis in the chicken choriallantoic membrane assay[22]. However, neither drug had any effect on bFGF-promoted proliferation, basal growth or survival of macrovascular and microvascular endothelial cells, smooth-muscle cells or Kaposi's-sarcoma cells (TABLE 2). But both drugs completely blocked bFGF-induced invasion of a reconstituted basement membrane (Matrigel) by all these cell types[22].

As tumour angiogenesis and invasion are general pathways involved in tumour progression, HIV-PIs might successfully be used to inhibit the growth of tumours of various origins and histotypes. In agreement with this hypothesis, indinavir and saquinavir also blocked tumour formation induced in nude mice by injection of EA-hy 926 cells — a hybrid between human endothelial cells and a lung adenocarcinoma cell line that is used as an angiogenic-tumour model[22]. Inhibition of these tumours was associated with inhibition of cell invasion, but not cell proliferation or cell survival[22]. Ongoing studies indicate that indinavir and saquinavir are also effective at inhibiting the growth of various human tumour xenografts, including lung, breast, hepatocarcinoma and colon adenocarcinoma, and human tumours of haematopoietic cell origin (myelomonocytic or T-cell acute leukaemia cells). These effects have also been shown to be mediated by a blockade of tumour angiogenesis and tumour-cell

HIV-1 Tat PROTEIN
A viral regulatory protein that induces expression of viral genes.

T_H1-TYPE PRO-INFLAMMATORY CYTOKINES
Cytokines produced by helper T cells of the T_H1 subset. These cytokines activate T cells and macrophages, and stimulate cell-mediated immunity. Most of these cytokines also have inflammatory activity, activate vessels and recruit immune cells at sites of tissue damage.

CLASSIC, AFRICAN AND POST-TRANSPLANT KAPOSI'S SARCOMA
Three epidemiological forms of the disease that share the same histopathological traits as HIV-associated Kaposi's sarcoma (AIDS-KS), although they are less aggressive, and are not associated with HIV infection. The classic form is prevalent in elderly people of Mediterranean origin, the African form is endemic in central Africa, and post-transplant Kaposi's sarcoma occurs in transplant patients receiving immune-suppressive therapy.

T-CELL PRIMING
Occurs following the first encounter of naive T cells with antigenic peptides on antigen-presenting cells, and results in the induction of a primary immune response.

CYTOTOXIC T LYMPHOCYTES
T cells that express the surface molecules CD3 and CD8, and recognize antigen peptides presented by MCH-class-I molecules. Following antigen recognition and stimulation by T helper cells, activation of these cells allows them to kill cancer cells and cells infected with intracellular pathogens such as viruses.

invasion, in the absence of effects on cell proliferation and viability (B.E., unpublished observations).

HIV-PIs exert other activities that might affect tumour-associated inflammation and tumour immunity in HIV-free models. For example, ritonavir and saquinavir inhibit the production and/or release of inflammatory cytokines and chemokines including TNFα, IL-6, and IL-8, by both peripheral-blood mononuclear cells and endothelial cells[130,133] (TABLE 3). This effect of HIV-PIs on inflammatory cytokines has been confirmed in treated patients, as PI–HAART has also been shown to inhibit TNFα, IL-2 and IFNγ production by peripheral-blood mononuclear cells from uninfected individuals who were treated with HIV-PIs for prophylactic intervention without acquiring HIV infection[134]. Similarly, ritonavir inhibits the expression by endothelial cells of adhesion molecules, including VCAM1, ICAM1, and selectin E, which are known to mediate leukocyte recruitment at sites of inflammation[130] (TABLE 3). These cytokines, chemokines and adhesion molecules are crucial in the development of Kaposi's sarcoma, as they mediate local inflammatory and immune responses to Kaposi's-sarcoma cells and to other KSHV-infected cells. Furthermore, they regulate survival, growth, invasion and eradication of most tumours. In fact, they lead to local stroma activation, basement-membrane and/or extracellular-matrix perturbation, and angiogenesis, and regulate local tumour immunity[135].

In this context, HIV-PIs directly modulate antigen processing, T-cell survival and proliferative responses[20,136–138], and they might even affect T-CELL PRIMING, as they can inhibit dendritic-cell maturation and function[139] (TABLE 3). In an *in vivo* model of infection by lymphocytic choriomeningitis virus, ritonavir has been shown to modify responses by CYTOTOXIC T LYMPHOCYTES (CTLs) to the virus by inhibiting the processing of viral antigens, although another study reported no alterations in the processing of commonly recognized HIV epitopes[20,140]. These data indicate that ritonavir might selectively modify the processing of certain antigens. So, ritonavir (and other HIV-PIs capable of affecting antigen processing) could have important effects on the generation of the tumour epitope repertoire, and therefore on the eradication of tumour cells by the immune system (TABLE 3).

Table 3 | **Indirect effects of HIV-PIs on tumours**

HIV-PI	Effect	Experimental model	Drug dose/ concentration	Mechanism(s) of action (molecular targets)	Potential antitumour action	References
Ritonavir	Inhibition of CTL responses	Injection into mouse footpad	HIV therapeutic dose	Modulation of CTL-epitope processing (proteasome subunits MB-1 and LMP7)	Evasion of CTL-mediated antitumour immune response	20,143
Ritonavir, saquinavir, indinavir, nelfinavir	Modulation of dendritic-cell maturation (that is, expression of co-stimulatory surface molecules) and function	Monocyte-derived dendritic cells	Similar to peak plasma concentration (10–20 μM)	Unknown (cell aspartic proteases, MMPs?)	Modulation of antitumour immunity	139
Ritonavir, saquinavir, indinavir	Inhibition of haematopoietic progenitor-cell and T-cell apoptosis (ritonavir); restoration of T-cell responsiveness to antigens (indinavir, saquinavir)	Cultured $CD34^+$ cells, T cells, PBMCs	Similar or below patient's steady-state plasma concentration (5 nM to 10 μM)	For ritonavir, decreased caspase expression or activity, and modulation of FAS receptor and ligand; mechanism unknown for indinavir and saquinavir (cell aspartic proteases, MMPs?)	Decreased tumour immune evasion	133,137, 138,141
Nelfinavir	Inhibition of T-cell apoptosis	Cultured T-cell line	Patient's peak plasma concentration (7 μM)	Inhibition of mitochondrial transmembrane potential loss (mitochondrial permeability transition-pore complex?)	Decreased tumour immune evasion	195
Ritonavir	Inhibition of IC production and endothelial-cell activation	Cultured endothelial cells	Below or similar to patient's peak plasma concentration (3–15 μM)	Inhibition of NF-κB activity (proteasome)	Inhibition of tumour-associated inflammation and stroma activation	130
Indinavir	Inhibition of IC production by PBMCs	Prophylactic antiretroviral therapy in HIV-uninfected individuals	PI–HAART doses	Inhibition of NF-κB activity (proteasome)	Inhibition of tumour-associated inflammation and stroma activation	134
Ritonavir	Reduction of endothelial-cell viability and proliferation	Cultured endothelial cells	Similar or above patient's peak plasma concentration (15–30 μM)	Cell necrosis (mitocondrial DNA?)	Blocks tumour angiogenesis	142

The potential antitumour effect of other human immunodeficiency virus protease inhibitors (HIV-PIs) used in the clinical practice, including amprenavir, lopinavir and atazanavir, is under study. CTL, cytotoxic T lymphocyte; HAART, highly active antiretroviral therapy; IC, inflammatory cytokine; MMP, matrix metalloproteinase; PBMC, peripheral-blood mononuclear cell.

Together, these data indicate that HIV-PIs directly block tumour development and progression as well as tumour metastasis by inhibiting tumour-cell survival, proliferation and invasion, and tumour angiogenesis, and by modulating immunity (FIG. 1).

Mechanisms of action

HIV-PIs differentially affect several tumour pathways, depending on the drug concentration used. For example, at concentrations similar to the lowest levels found in plasma of treated patients (that is, 0.1–1.0 μM), saquinavir and indinavir effectively block invasion of a basement membrane (Matrigel) by endothelial and Kaposi's-sarcoma cells, as well as by lung, breast, colon adenocarcinoma, and myelo-monocytic and T-cell acute leukaemia cells, with no effects on cell survival or proliferation (REF. 22 and B.E., unpublished observations). At drug concentrations above the therapeutic peak level (50–100 μM), however, saquinavir causes high levels of apoptosis in tumour cells of several tumour types, including prostate cancer, lymphoblastoid leukaemia and glioblastoma[21]. Both indinavir and saquinavir, used at drug concentrations that are too low to achieve consistent HIV inhibition (10^{-2}–10^{-1} μM), increase the survival of T cells isolated from HIV-infected patients, and restore antigen-specific proliferative responses to these cells[137]. Similarly, ritonavir decreases the rate of apoptotic cell death in T cells and

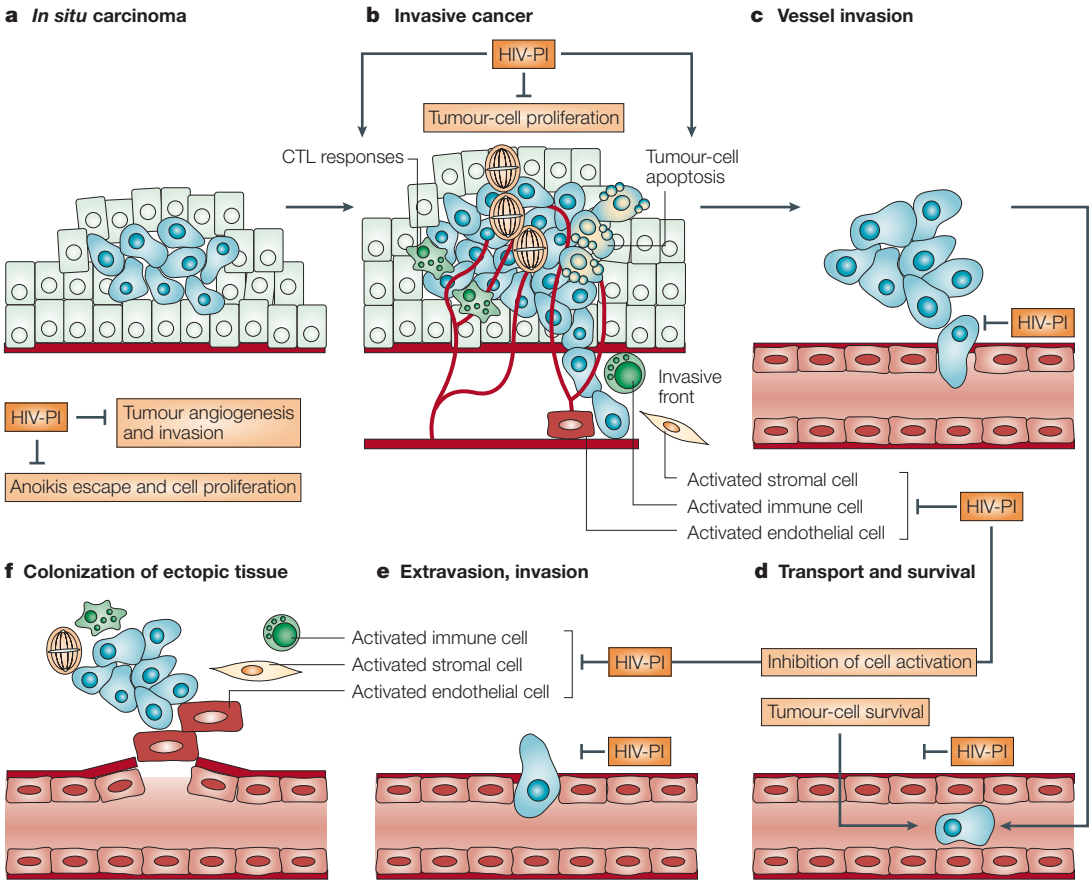


Figure 1 | **Steps in tumour progression and metastasis affected by HIV-protease inhibitors.** Tumour pathways that underlie the various steps of cancer development can be disrupted by human immunodeficiency virus protease inhibitors (HIV-PIs). These steps usually lead to progression of *in situ* carcinoma (**a**) to invasive cancer (**b**) and to metastasis formation and dissemination (**c**–**f**). Tumour outgrowth (**a**,**b**) is dependent on tumour neoangiogenesis and its net rate is determined by the balance between tumour-cell proliferation versus apoptosis, invasive behaviour and the ability of tumour cells to evade the immune response. At concentrations similar or above therapeutic peak levels, HIV-PIs promote apoptosis and inhibit proliferation of tumour cells with little or no effects on survival and proliferation of normal cells, whereas at therapeutic steady-state concentrations they inhibit tumour angiogenesis and tumour-cell invasion. Furthermore, HIV-PIs have anti-inflammatory effects (**b**). As metastatic cell clones emerge, tumour cells loosen their contact with surrounding cells and the extracellular matrix (ECM). This leads to invasion of blood or lymphatic vessels and to extravasation of tumour cells at distant sites (**c**–**e**). These steps require the degradation of basement membranes and, at the same time, inhibition of apoptosis following loss of cell anchorage (anoikis) — processes that are also inhibited by HIV-PIs. Finally, colonization of ectopic tissue by tumour cells (**f**) is required for establishment of metastases, and this process is similarly affected by HIV-PIs. During tissue invasion and establishment of metastases (**b**,**f**), activated endothelial cells, stromal cells and immune cells cooperate in basement membrane and ECM degradation, modify the ECM composition, release ECM-bound growth and angiogenic factors, and produce cytokines and chemokines that stimulate tumour-cell growth and migration, and recruit all these cell types at the invasive front. These processes are all affected by HIV-PIs through their ability to inhibit cytokine and chemokine production, cell activation, and basal membrane and ECM degradation and remodelling.

in CD34$^+$ haematopoietic-cell progenitors from HIV-non-infected individuals when used at very low drug concentrations (5×10^{-3} to 10^{-2} μM), an effect that is most likely mediated by a decrease in the expression of both caspases and FAS ligand[133,138,141]. By contrast, at higher concentrations (5–10 μM) indinavir and ritonavir block T cells from both HIV-infected and non-infected individuals in the G_0/G_1 or G_1/S phase of the cell cycle, and induce necrotic or apoptotic death of T cells and endothelial cells[129,136,138,141,142].

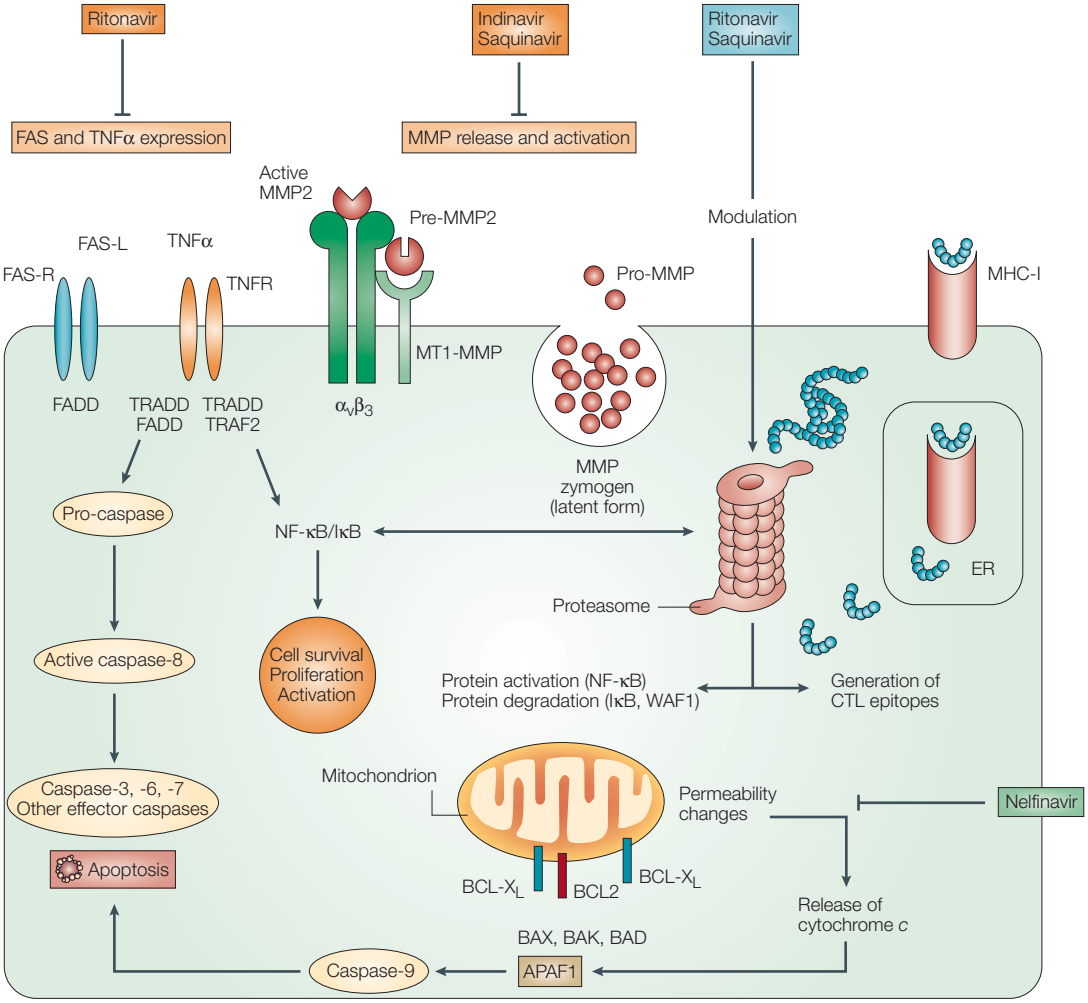


Figure 2 | **Molecular targets of antiviral agents.** Human immunodeficiency virus protease inhibitors (HIV-PIs) block several signalling pathways that regulate processes such as cell proliferation, survival, activation and invasion. Ritonavir inhibits the expression of FAS ligand (FAS-L) and tumour-necrosis factor-α (TNFα). Signalling by these molecules normally occurs through their receptors (FAS-R, which is also downregulated by ritonavir, and TNF-R, respectively) and downstream molecules, such as FADD and TRADD, to activate caspases (caspases-3, -6 and -7) that induce cell death. TRAF2, however, is also activated by TNFα, which promotes cell survival and proliferation through NF-κB. Inhibition of FAS-R and FAS-L would promote survival of activated leukocytes, which might prevent tumour immune evasion. By contrast, inhibition of TNFα production by activated lymphocytes[130] might inhibit NF-κB activation through TRAF2, inhibiting tumour-cell survival. Both ritonavir and saquinavir inhibit or modulate 26S proteasome activity, preventing IκB degradation and thereby blocking NF-κB activation. Ritonavir has also been shown to block NF-κB activation in response to inflammatory cytokines (TNFα signalling) and viral products, including the HIV-1 Tat protein or the gene product of Kaposi's-sarcoma-associated herpesvirus *ORF74*, which are known to contribute to Kaposi's sarcoma[130]. Through these actions, these HIV-PIs inhibit tumour-cell survival and proliferation. The ability of these drugs to alter 26S proteasome activity might also modify antigen processing, inhibiting the formation of certain peptide epitopes for presentation to cytotoxic T lymphocytes (CTLs)[20,143]. These epitopes are normally transported through the endoplasmic reticulum (ER) and presented to CTLs by MHC-class-I (MHC-I) molecules. Alterations in this process could prevent tumour-cell immune evasion by altering the tumour-epitope immunodominance hierarchy. Nelfinavir inhibits apoptosis by preventing loss of mitochondrial-membrane potential and by stabilizing membrane permeability. This prevents the release of cytochrome *c* and activation other pro-apoptotic molecules, such as apoptotic protease activating factor 1 (APAF1), BAX, BAK, BAD and caspase-9. At low concentrations, saquinavir and indinavir block matrix metalloproteinase 2 (MMP2) proteolytic activation, which requires the binding of MMP2 to integrin[22]. By contrast, these drugs do not affect the MMP2 pre-activation proteolytic step, which is catalysed by a membrane-associated MMP — membrane type 1 MMP (MT1-MMP). Saquinavir can also inhibit the production and/or release of MMP zymogen (pro-MMP). These effects of indinavir and saquinavir can block angiogenesis and tumour-cell invasion, and might also affect cell survival and inflammation.

Proteasome inhibition. How could protease inhibitors mediate all these antitumour effects and affect all these cell pathways (FIG. 2)? These drugs have been shown to function by inhibiting the activity of the proteasome. This effect occurs at a relatively wide range of drug concentrations (5–100 μM), which are similar to, or above, the pharmacokinetic peak level present in the sera of treated patients[20,21,129,130,143–145]. Within this range, ritonavir, indinavir and saquinavir have been shown not only to inhibit, to various degrees, tryptic, chymotryptic and peptidyl-glutamyl activity of isolated 26S and/or 20S proteasome complexes, but also the function of the 26S proteasome in cultured cells, although for indinavir these effects require combination with NRTIs[20,21,129,130,143–145]. The proteasome controls several cell pathways, including protein turnover, clearance of misfolded proteins, apoptosis, degradation of tumour-suppressor gene products, the function of cyclin-dependent kinase (CDK) inhibitors, and the proteolytic maturation and activation of the transcription factor NF-κB[146–148]. The ability of HIV-PIs to block these activities can inhibit tumour-cell survival and proliferation, as well as tumour-associated inflammation and endothelial-cell activation. By inhibiting the proteasome, HIV-PIs can also sensitize tumour cells to ionizing radiation[146–149]. Studies have shown that HIV-PIs act as reversible proteasome inhibitors[129,150], much like some potent proteasome inhibitors already being tested in *in vitro* preclinical studies and in clinical trials as anticancer agents, such as bortezomib or TMC-95 (REFS 147,151).

For example, saquinavir reversibly inhibits the degradation of ubiquitylated proteins and, through this activity, reversibly sensitizes prostatic tumour cells to ionizing radiation[21]. Ritonavir, in turn, modulates protein turnover by selectively and reversibly inhibiting the degradation of some, but not all, proteasome substrates, particularly non-ubiquitylated proteins, such as the CDK inhibitor WAF1 (REFS 129,143) (FIG. 2). These actions of ritonavir are due to its capability of selectively binding the MB-1 and LMP7 proteasome subunits[143]. As LMP7 replaces MB-1 after the generation of the IMMUNOPROTEASOME during inflammatory processes (an effect that is mediated by IFNγ)[152], these findings could also explain the capacity of ritonavir to modulate antigen processing and to suppress CTL-dependent inflammatory responses in mice infected by the lymphocytic choriomeningitis virus[20]. Furthermore, as immunoproteasomes are particularly efficient at generating CTL-epitope peptides, and are constitutively present in PROFESSIONAL ANTIGEN-PRESENTING CELLS, it is possible that HIV-PIs modify the epitope repertoire of tumour antigens. This would have important implications for tumour immunity and tumour control and/or eradication by the immune system (FIG. 2). Although the drug concentrations required to elicit these effects on the proteasome are often well above the pharmacokinetic peak concentration present in treated patients, proteasome modulation at therapeutic drug concentrations could be effective when HIV-PIs are used in combination with NRTIs, such as in HAART regimens. In fact, indinavir has been shown inhibit the 26S proteasome when combined with the NRTIs zidovudine and lamivudine[145]. Furthermore, inhibition of the proteasome by HIV-PIs has been implicated in the reduced degradation of apolipoprotein B, which, in turn, is associated with HAART-induced hyperlipidaemia[153]. All these data indicate that HIV-PIs could affect proteasome activity in treated patients.

MMP inhibition. A different mechanism of action, however, is observed at drug concentrations that are too low to affect the cell proteasome, but are similar to the lowest peak level present in the sera from treated patients (0.1–1.0 μM)[22]. At these lower concentrations, HIV-PIs, including indinavir and saquinavir, inhibit angiogenesis and cell invasion through their effects on the activity of MMPs, particularly MMP2 (REF. 22). MMPs, or the enzymes and molecules known to regulate their production and/or activity, do not share any sequence homology or structural similarity with the HIV protease[22]. Furthermore, HIV protease and MMPs do not belong to the same functional class of proteases[22]. Accordingly, indinavir and saquinavir do not affect the catalytic activity of MMPs in a direct way[22]. Rather, they inhibit the proteolytic activation of MMP2 with no effects on its production or release[22].

MMP2 activation is initiated by proteolytic enzymes, such as membrane type 1 MMP (MT1-MMP), which cleave latent MMP2 to produce the pre-active form of the enzyme (pre-MMP2). In turn, pre-MMP2 is fully activated after autoproteolytic cleavage that is mediated by the $\alpha_v\beta_3$-integrin[22]. The exact mechanism for the inhibition of MMP2 activation by HIV-PIs (and whether integrins are direct targets of HIV-PIs) has not yet been determined. However, *in vitro* studies indicate that activation of purified MMP2 is not affected by HIV-PIs, indicating a role for MT1-MMP, $\alpha_v\beta_3$-integrin and/or other molecules involved in MMP production or activation in this effect of HIV-PI (B.E., unpublished observations). Furthermore, HIV-PIs affect the production and/or release, but not the activation, of MMP3 (REF. 154). As MMP production is induced after integrin activation[155,156], it is possible that integrins or molecules involved in integrin signalling could be targeted by HIV-PIs.

This effect of HIV-PIs on MMPs, cell invasion and angiogenesis is likely to inhibit tumour growth and metastasis (FIGS 1,2), as well as lead to the regression of early tumours, with little effect on already established or advanced tumours. In fact, neoformed and yet non-stabilized vessels, which are the targets of anti-angiogenic agents, are prominent in early but not in advanced tumours. Indeed, PI–HAART promotes regression of Kaposi's sarcoma and prolongs disease-free time in patients with early disease, whereas such responses are only achieved in patients with massive, advanced disease after debulking chemotherapy (REF. 157 and U. Tirelli, personal communication).

Furthermore, MMPs are involved in several crucial immune and immunomodulatory functions, and in cancer-mediated immune suppression. Evidence indicates, in fact, that MMPs participate in antigen

IMMUNOPROTEASOME
A form of proteasome that is present in professional antigen-presenting cells and is induced in other cell types by inflammation. The immunoproteasome produces epitope peptides for presentation to cytotoxic T cells.

PROFESSIONAL ANTIGEN-PRESENTING CELLS
Cells that internalize and process antigens for presentation to immune cells by MHC-class-I and -II molecules. Include macrophages, B cells and dendritic cells.

Table 4 | **Clinical studies/trials underway to evaluate the effects of HIV-PIs on cancer**

Clinical setting	Type of study	Treatment	End points
Tumour-free HIV-infected patients	Retrospective	PI–HAART versus NNRTI–HAART	Determination of serum levels of MMPs, angiogenic factors, cytokines and immune-activation molecules
HIV-infected patients with or without KS or NHL	Prospective	PI–HAART plus/ minus chemotherapy	Determination of the serum levels of MMPs, angiogenic factors, cytokines, and immune-activation molecules
HIV-infected patients with early-stage KS	Retrospective/ prospective (Phase II)	PI–HAART versus NNRTI–HAART	Evaluation of clinical response and determination of serum levels of MMPs, angiogenic factors, inflammatory cytokines and immune-activation molecules
HIV-uninfected patients with early and advanced classical KS	Prospective (Phase II)	Indinavir alone	Evaluation of clinical response, safety and tolerability to indinavir; determination of markers of angiogenesis (serum levels of MMPs, bFGF, VEGF), immune activation (soluble CD8, neopterin, IFNγ and IL-5 serum levels) and endothelial-cell activation (soluble ICAM1 serum levels); humoral and cellular responses to KSHV and ubiquitous viruses (CMV, EBV, influenza), and recall antigens (tetanus, candida, tuberculosis)
HIV-uninfected patients with advanced classical KS	Prospective (Phase II)	Indinavir plus chemotherapy	Evaluation of clinical response, safety and tolerability to indinavir plus chemotherapy in the induction and maintenance phase; determination of serum levels of MMPs and other surrogate markers of angiogenesis, markers of immune (cytokine levels) and endothelial-cell activation (soluble ICAM1 serum levels)
HIV-infected patients with NHL	Prospective (Phase II)	Rituximab plus chemotherapy and PI–HAART versus NNRTI–HAART	Evaluation of clinical response and determination of serum levels of MMPs,angiogenic factors, inflammatory cytokines and immune-activation molecules
HIV-uninfected patients with advanced non-small-cell lung carcinoma	Prospective (Phase II)	Indinavir plus platin-containing chemotherapy	Clinical response; determination of serum levels of MMPs and angiogenic factors

bFGF, basic fibroblast growth factor; CMV, cytomegalovirus; EBV, Epstein–Barr virus; HAART, highly active antiretroviral therapy; HIV-PI, human immunodeficiency virus protease inhibitor; ICAM1, intercellular adhesion molecule 1; IFNγ, interferon-γ; IL-5, interleukin-5; KS, Kaposi's sarcoma; KSHV, Kaposi's-sarcoma-associated herpesvirus; MMP, matrix metalloproteinase; NHL, non-Hodgkin's lymphoma; NNRTI, non-nucleoside reverse-transcriptase inhibitor; VEGF, vascular endothelial growth factor.

processing[158]. In addition, recent studies have shown that the direct injection of recombinant MMPs in mice can induce dendritic-cell maturation and trafficking[159]. Moreover, MMPs, including MMP2, act as potent modulators of local inflammation by activating or degrading inflammatory cytokines and chemokines present on the cell membrane, such as TNFα, IL-1β, monocyte chemoattractant protein 3 and IL-8 (REFS 160–164). Importantly, MMPs activate transforming growth factor-β, which, in turn, inhibits T-cell responses against tumours[165,166]. MMPs can also cleave IL-2 receptor-α[167], which is required for T-cell proliferation following antigen stimulation. These effects of MMPs are important determinants of tumour immune evasion, but might also explain the strong stimulatory effect of low HIV-PI concentrations on T-cell proliferation and survival. Furthermore, as MMPs are required for leukocyte transmigration and tissue infiltration by inflammatory cells, the capability of ritonavir to inhibit CTL-dependent inflammatory responses could be mediated not only following the modulation of CTL-epitope processing by the proteasome, but also through the inhibition of MMP activation or function in transmigrating lymphocytes[140]. Together, these actions on MMPs could explain the ability of PI–HAART to inhibit T-cell activation, inflammatory cytokine production, and inflammation, as well as to modulate dendritic-cell maturation, and might contribute to their antitumour effects in patients.

As HIV-PIs are cleared with very fast kinetics in patients, plasma concentrations of these drugs are only transiently sufficient to affect proteasome function, but remain for long periods of time above the concentration required to block MMPs, cell invasion and angiogenesis. So, blockage of cell invasion and angiogenesis through MMP inhibition is likely to be the most prominent mechanism underlying the antitumour effects of HIV-PIs in patients[22]. Studies have indicated that PI–HAART inhibits MMPs in humans, as mutations in genes encoding MMP2 or MT1-MMP lead to bone disorders such as OSTEOPAENIA, increased bone resorption and OSTEOPOROSIS[168] — effects that are also observed in patients who are treated with PI–HAART[169,170].

Future directions

Based on what we have learned about the mechanisms of HIV-PIs, might these drugs be exploited for the therapy of patients with non-HIV-associated cancers? The actions of HIV-PIs on cell survival, apoptosis, cell invasion, angiogenesis, inflammation and immune responses indicate that these drugs could have widespread use in the treatment of all cancer types. In particular, HIV-PIs should be exploited, alone or in combination with conventional cytotoxic therapy, for the treatment of tumours that have been shown to be sensitive to anti-angiogenic therapy, such as colon and renal cancer, and multiple myeloma[171], or to prevent the invasion of pre-malignant diseases, such as *in situ* carcinomas of the oral cavity and cervix. In addition,

OSTEOPAENIA AND OSTEOPOROSIS
Bone undergoes continuous remodelling through resorption and formation of the calcified matrix. Osteopaenia (bone thinning) and osteoporosis (bone softening) are characterized by bone demineralization, leading to low bone mineral density or content.

HIV-PIs might have a favourable therapeutic index in the treatment of tumours that are sensitive to the anti-inflammatory cyclooxygenase (COX) inhibitors, such as gastrointestinal, breast and lung cancer[172,173]. In fact, COX2 is induced by inflammatory cytokines that, in turn, are inhibited by HIV-PIs[173]. Because of their anti-angiogenic and pro-apoptotic actions, HIV-PIs might be useful in preventing tumour recurrence or metastasis after conventional radiation or cytotoxic therapy. This is also indicated by the increased survival of patients with AIDS-associated tumours treated with HAART combined with chemotherapy[174].

It is, therefore, important to determine the specific interactions of HIV-PIs with conventional cytotoxic drugs. In recent studies, PI–HAART has not shown significant interactions with doxorubicin or paclitaxel[175,176], but it has been reported to increase the haematological and neurological toxicity of chemotherapy[177,178]. This, however, could be due to NRTIs, which are known to exert such toxic effects[177]. In this context, HIV-PIs have been shown to inhibit apoptosis of haematopoietic precursors and polymorphonuclear leukocytes (PMNL) and to stimulate haematopoiesis and PMNL function[141,179–181]. So, when used in uninfected individuals, HIV-PIs might decrease, rather than increase, the haemotological side effects of cytotoxic drugs. In addition, as ritonavir inhibits the functional activity of multidrug-resistance-related protein 1, it might be exploited to overcome resistance to anticancer drugs[182].

The relatively low toxicity of these drugs and the large body of data on their pharmacokinetics, tissue distribution and drug interactions would allow for the rapid clinical evaluation of HIV-PIs alone or combined with other molecules in patients with cancer. To this end, a Phase II clinical trial for the treatment of classical Kaposi's sarcoma in HIV-uninfected individuals with indinavir, and other clinical studies for the treatment of other tumour types with HIV-PIs, are underway in Italy to asses the efficacy of HIV-PIs, alone or in combination with classic cytotoxic drugs, in HIV-positive and HIV-negative cancer patients (TABLE 4). Surrogate markers of angiogenesis, tumour invasion, immune activation, and T_H1 and T_H2 responses could be used to validate the actions of these drugs in patients (TABLE 4). It is also of great importance to better dissect the anti-angiogenic and antitumour actions of the new NNRTI-based regimens, compared with PI–HAART, to determine if these will be as effective in the management of HIV-infected patients with tumours or who are at risk of tumours. Further basic and preclinical studies to compare the antitumour activity of NNRTIs versus HIV-PIs in non-HIV-associated models are needed, as well as retrospective and prospective studies aimed at dissecting the non-antiretroviral actions of NNRTIs in HIV-infected patients (TABLE 4). Finally, a complete exploitation of HIV-PIs as antitumour drugs requires further definition of their exact mechanism of action. The integration of functional genomics and proteomics techniques in the study of the effect of HIV-PIs on tumour behaviour; molecular modelling and docking approaches; and protein–ligand interaction studies are required to identify the exact targets of HIV-PIs, and for developing new HIV-PI derivatives and analogues with higher therapeutic indexes.

Recent studies have indicated that the proteasome might regulate angiogenesis and cell invasion by modulating MMP expression and/or activation as well as angiogenic-factor receptors[183–186]. So, there might be crosstalk between the two key pathways that are targeted by HIV-PIs. Studying the effects of HIV-PIs on tumour cells could shed light on such a connection, leading to new avenues in drug discovery based on the rational design of a new class of antitumour drugs based on HIV-PIs.

1. Munoz, A. *et al.* Trends in the incidence of outcomes defining acquired immunodeficiency syndrome (AIDS) in the Multicenter AIDS Cohort Study: 1985–1991. *Am. J. Epidemiol.* **137**, 1985–1991 (1993).
2. Detels, R. *et al.* Effectiveness of potent antiretroviral therapy on time to AIDS and death in men with known HIV infection duration. Multicenter AIDS Cohort Study Investigators. *JAMA* **280**, 1497–1503 (1998).
3. Pomerantz, R. J. & Horn, D. L. Twenty years of therapy for HIV-1 infection. *Nature Med.* **9**, 867–873 (2003).
4. Autran, B. *et al.* Positive effects of combined antiretroviral therapy on $CD4^+$ T cell homeostasis and function in advanced HIV disease. *Science* **277**, 112–116 (1997).
5. Douek, D. C. *et al.* Changes in thymic function with age and during the treatment of HIV infection. *Nature* **396**, 690–695 (1998).
6. Gulick, R. M. *et al.* Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. *N. Engl. J. Med.* **337**, 734–739 (1997).
7. Gulick, R. M. *et al.* 3-year suppression of HIV viremia with indinavir, zidovudine, and lamivudine. *Ann. Intern. Med.* **133**, 35–39 (2000).
8. International Collaboration on HIV and Cancer. Highly active antiretroviral therapy and incidence of cancer in human immunodeficiency virus-infected adults. *J. Natl. Cancer Inst.* **92**, 1823–1830 (2000).
A large survey analysing tumour incidence in 48,000 HIV-infected individuals from 23 cohort studies, comparing incidence rates in years before the advent of HAART (1992–1996) to rates in years after the widespread use of HAART (1997–1999).
9. Heard, I., Schmitz, V., Costagliola, D., Orth, G. & Kazatchkine, M. D. Early regression of cervical lesions in HIV-seropositive women receiving highly active antiretroviral therapy. *AIDS* **12**, 1459–1464 (1998).
10. Mo, Y. *et al.* Simultaneous targeting of telomeres and telomerase as a cancer therapeutic approach. *Cancer Res.* **63**, 579–585 (2003).
11. Ghosh, S. K. *et al.* Potentiation of TRAIL-induced apoptosis in primary effusion lymphoma through azidothymidine-mediated inhibition of NF-κB. *Blood* **101**, 2321–2327 (2003).
12. Morgan, R. J. Jr *et al.* Phase I study of cisdiamminedichloroplatinum in combination with azidothymidine in the treatment of patients with advanced malignancies. *Cancer Chemother. Pharmacol.* **51**, 459–464 (2003).
13. Rarick, M. U. *et al.* Treatment of epidemic Kaposi's sarcoma with combination chemotherapy (vincristine and bleomycin) and zidovudine. *Ann. Oncol.* **1**, 147–149 (1990).
14. Portsmouth, S. *et al.* A comparison of regimens based on non-nucleoside reverse transcriptase inhibitors or protease inhibitors in preventing Kaposi's sarcoma. *AIDS* **17**, F17–F22 (2003).
15. Rabkin, C. S., Testa, M. A., Huang, J. & Von Roenn, J. H. Kaposi's sarcoma and non-Hodgkin's lymphoma incidence trends in AIDS Clinical Trial Group study participants. *J. Acquir. Immune Defic. Syndr.* **21**, S31–S33 (1999).
16. Stebbing, J. *et al.* Antiretroviral treatment regimens and immune parameters in the prevention of systemic AIDS-related non-Hodgkin's lymphoma. *J. Clin. Oncol.* **22**, 2177–2183 (2004).
17. Aaron, L., Lidove, O., Yousry, C., Dupont, B. & Viard, J. P. Human herpesvirus 8-positive Castleman disease in human immunodeficiency virus-infected patients: the impact of highly active antiretroviral therapy. *Clin. Infect. Dis.* **35**, 880–882 (2002).
18. Carre, D. *et al.* Epidermodysplasia verruciformis in a patient with HIV infection: no response to highly active antiretroviral therapy. *Int. J. Dermatol.* **42**, 296–300 (2003).
19. Kirk, O. *et al.* Non-Hodgkin lymphoma in HIV-infected patients in the era of highly active antiretroviral therapy. *Blood* **98**, 3406–3412 (2001).
An extensive retrospective multicentric study identifying a significant decrease in all NHL subtypes in patients treated with HAART compared with patients treated with non-HAART regimens, or to untreated patients. Importantly, this study shows an additional decreased risk of NHL in patients who did not respond to HAART therapy.
20. Andre, P. *et al.* An inhibitor of HIV-1 protease modulates proteasome activity, antigen presentation, and T cell responses. *Proc. Natl Acad. Sci. USA* **95**, 13120–13124 (1998).
Shows for the first time that HIV-PIs can affect cancer-related host-cell pathways in HIV-free models, both *in vitro* and *in vivo*. This paper has lead to the concept that PI–HAART might affect tumour immunity.
21. Pajonk, F., Himmelsbach, J., Riess, K., Sommer, A. & McBride, W. H. The human immunodeficiency virus (HIV)-1 protease inhibitor saquinavir inhibits proteasome function and causes apoptosis and radiosensitization in non-HIV-

associated human cancer cells. *Cancer Res.* **62**, 5230–5235 (2002).
Shows that, due to actions on the cell proteasome, PI–HAART might cooperate with radiotherapy in tumour eradication.
22. Sgadari, C. *et al.* HIV protease inhibitors are potent anti-angiogenic molecules and promote regression of Kaposi sarcoma. *Nature Med.* **8**, 225–232 (2002).
The authors used non-HIV infected *in vitro* and *in vivo* models to show that HIV-PIs inhibit angiogenesis and tumour growth by blocking MMP2 activation.
23. Ensoli, B., Sturzl, M. & Monini, P. Reactivation and role of HHV-8 in Kaposi's sarcoma initiation. *Adv. Cancer Res.* **81**, 161–200 (2001).
24. Chun, T. W., Chadwick, K., Margolick, J. & Siliciano, R. F. Differential susceptibility of naive and memory CD4$^+$ T cells to the cytopathic effects of infection with human immunodeficiency virus type 1 strain LAI. *J. Virol.* **71**, 4436–4444 (1997).
25. Schnittman, S. M. *et al.* Preferential infection of CD4$^+$ memory T cells by human immunodeficiency virus type 1: evidence for a role in the selective T-cell functional defects observed in infected individuals. *Proc. Natl Acad. Sci. USA* **87**, 6058–6062 (1990).
26. Engels, E. A. *et al.* Detection and quantification of Kaposi's sarcoma-associated herpesvirus to predict AIDS-associated Kaposi's sarcoma. *AIDS* **17**, 1847–1851 (2003).
27. Mbulaiteye, S. M., Biggar, R. J., Goedert, J. J. & Engels, E. A. Immune deficiency and risk for malignancy among persons with AIDS. *J. Acquir. Immune. Defic. Syndr.* **32**, 527–533 (2003).
Describes the relationship between CD4$^+$ T-cell loss and tumour risk in HIV-infected individuals, pointing to an immune-deficiency threshold for certain types of cancers.
28. Rezza, G. *et al.* Human herpesvirus 8 seropositivity and risk of Kaposi's sarcoma and other acquired immunodeficiency syndrome-related diseases. *J. Natl. Cancer Inst.* **91**, 1468–1474 (1999).
29. Gao, S. J. *et al.* Seroconversion to antibodies against Kaposi's sarcoma-associated herpesvirus-related latent nuclear antigens before the development of Kaposi's sarcoma. *N. Engl. J. Med.* **335**, 233–241 (1996).
30. Malnati, M. *et al.* Retrospective analysis of HHV-8 viremia and cellular viral load in HIV-seropositive patients receiving interleukin 2 in combination with antiretroviral therapy. *Blood* **100**, 1575–1578 (2002).
31. Quinlivan, E. B. *et al.* Elevated virus loads of Kaposi's sarcoma-associated human herpesvirus 8 predict Kaposi's sarcoma disease progression, but elevated levels of human immunodeficiency virus type 1 do not. *J. Infect. Dis.* **185**, 1736–1744 (2002).
32. Whitby, D. *et al.* Detection of Kaposi sarcoma associated herpesvirus in peripheral blood of HIV-infected individuals and progression to Kaposi's sarcoma. *Lancet* **346**, 799–802 (1995).
33. Kersten, M. J., Klein, M. R., Holwerda, A. M., Miedema, F. & van Oers, M. H. Epstein–Barr virus-specific cytotoxic T cell responses in HIV-1 infection: different kinetics in patients progressing to opportunistic infection or non-Hodgkin's lymphoma. *J. Clin. Invest.* **99**, 1525–1533 (1997).
34. van Baarle, D. *et al.* Dysfunctional Epstein–Barr virus (EBV)-specific CD8$^+$ T lymphocytes and increased EBV load in HIV-1 infected individuals progressing to AIDS-related non-Hodgkin lymphoma. *Blood* **98**, 146–155 (2001).
35. van Baarle, D. *et al.* Lack of Epstein–Barr virus- and HIV-specific CD27-CD8$^+$ T cells is associated with progression to viral disease in HIV-infection. *AIDS* **16**, 2001–2011 (2002).
36. Davi, F. *et al.* Burkitt-like lymphomas in AIDS patients: characterization within a series of 103 human immunodeficiency virus-associated non-Hodgkin's lymphomas. Burkitt's Lymphoma Study Group. *J. Clin. Oncol.* **16**, 3788–3795 (1998).
37. Pedersen, C. *et al.* HIV-associated lymphoma: histopathology and association with Epstein–Barr virus genome related to clinical, immunological and prognostic features. *Eur. J. Cancer* **27**, 1416–1423 (1991).
38. Pluda, J. M. *et al.* Parameters affecting the development of non-Hodgkin's lymphoma in patients with severe human immunodeficiency virus infection receiving antiretroviral therapy. *J. Clin. Oncol.* **11**, 1099–1107 (1993).
39. Roithmann, S., Tourani, J. M. & Andrieu, J. M. AIDS-associated non-Hodgkin lymphoma. *Lancet* **338**, 884–885 (1991).
40. Skiest, D. J. & Crosby, C. Survival is prolonged by highly active antiretroviral therapy in AIDS patients with primary central nervous system lymphoma. *AIDS* **17**, 1787–1793 (2003).
41. Tirelli, U. *et al.* Hodgkin's disease and human immunodeficiency virus infection: clinicopathologic and virologic features of 114 patients from the Italian Cooperative Group on AIDS and Tumors. *J. Clin. Oncol.* **13**, 1758–1767 (1995).
42. Grossman, Z., Meier-Schellersheim, M., Sousa, A. E., Victorino, R. M. & Paul, W. E. CD4$^+$ T-cell depletion in HIV infection: are we closer to understanding the cause? *Nature Med.* **8**, 319–323 (2002).
An important overview highlighting the role of immune activation in T-cell losses in HIV infection.
43. Hellerstein, M. K. *et al.* Subpopulations of long-lived and short-lived T cells in advanced HIV-1 infection. *J. Clin. Invest.* **112**, 956–966 (2003).
44. Kovacs, J. A. *et al.* Identification of dynamically distinct subpopulations of T lymphocytes that are differentially affected by HIV. *J. Exp. Med.* **194**, 1731–1741 (2001).
45. Ribeiro, R. M., Mohri, H., Ho, D. D. & Perelson, A. S. *In vivo* dynamics of T cell activation, proliferation, and death in HIV-1 infection: why are CD4$^+$ but not CD8$^+$ T cells depleted? *Proc. Natl Acad. Sci. USA* **99**, 15572–15577 (2002).
46. Fiorelli, V., Gendelman, R., Samaniego, F., Markham, P. D. & Ensoli, B. Cytokines from activated T cells induce normal endothelial cells to acquire the phenotypic and functional features of AIDS-Kaposi's sarcoma spindle cells. *J. Clin. Invest* **95**, 1723–1734 (1995).
47. Fiorelli, V. *et al.* IFN-γ induces endothelial cells to proliferate and to invade the extracellular matrix in response to the HIV-1 Tat protein: implications for AIDS-Kaposi's sarcoma pathogenesis. *J. Immunol.* **162**, 1165–1170 (1999).
48. Barillari, G. *et al.* Inflammatory cytokines synergize with the HIV-1 Tat protein to promote angiogenesis and Kaposi's sarcoma via induction of basic fibroblast growth factor and the αvβ3 integrin. *J. Immunol.* **163**, 1929–1935 (1999).
49. Barillari, G. *et al.* The Tat protein of human immunodeficiency virus type-1 promotes vascular cell growth and locomotion by engaging the α5β1 and αvβ3 integrins and by mobilizing sequestered basic fibroblast growth factor. *Blood* **94**, 663–672 (1999).
50. Ensoli, B. *et al.* Synergy between basic fibroblast growth factor and HIV-1 Tat protein in induction of Kaposi's sarcoma. *Nature* **371**, 674–680 (1994).
51. Chang, H. C., Samaniego, F., Nair, B. C., Buonaguro, L. & Ensoli, B. HIV-1 Tat protein exits from cells via a leaderless secretory pathway and binds to extracellular matrix-associated heparan sulfate proteoglycans through its basic region. *AIDS* **11**, 1421–1431 (1997).
52. Ensoli, B., Barillari, G., Salahuddin, S. Z., Gallo, R. C. & Wong-Staal, F. Tat protein of HIV-1 stimulates growth of cells derived from Kaposi's sarcoma lesions of AIDS patients. *Nature* **345**, 84–86 (1990).
53. Toschi, E. *et al.* Activation of matrix-metalloproteinase-2 and membrane-type-1-matrix-metalloproteinase in endothelial cells and induction of vascular permeability *in vivo* by human immunodeficiency virus-1 Tat protein and basic fibroblast growth factor. *Mol. Biol. Cell* **12**, 2934–2946 (2001).
54. Browning, P. J. *et al.* Identification and culture of Kaposi's sarcoma-like spindle cells from the peripheral blood of human immunodeficiency virus-1-infected individuals and normal controls. *Blood* **84**, 2711–2720 (1994).
55. Fiorelli, V. *et al.* γ-Interferon produced by CD8$^+$ T cells infiltrating Kaposi's sarcoma induces spindle cells with angiogenic phenotype and synergy with human immunodeficiency virus-1 Tat protein: an immune response to human herpesvirus-8 infection? *Blood* **91**, 956–967 (1998).
56. Monini, P. *et al.* Reactivation and persistence of human herpesvirus-8 infection in B cells and monocytes by Th-1 cytokines increased in Kaposi's sarcoma. *Blood* **93**, 4044–4058 (1999).
57. Sirianni, M. C. *et al.* γ-Interferon production in peripheral blood mononuclear cells and tumor infiltrating lymphocytes from Kaposi's sarcoma patients: correlation with the presence of human herpesvirus-8 in peripheral blood mononuclear cells and lesional macrophages. *Blood* **91**, 968–976 (1998).
58. Samaniego, F., Markham, P. D., Gallo, R. C. & Ensoli, B. Inflammatory cytokines induce AIDS-Kaposi's sarcoma-derived spindle cells to produce and release basic fibroblast growth factor and enhance Kaposi's sarcoma-like lesion formation in nude mice. *J. Immunol.* **154**, 3582–3592 (1995).
59. Samaniego, F. *et al.* Vascular endothelial growth factor and basic fibroblast growth factor present in Kaposi's sarcoma (KS) are induced by inflammatory cytokines and synergize to promote vascular permeability and KS lesion development. *Am. J. Pathol.* **152**, 1433–1443 (1998).
60. Samaniego, F., Markham, P. D., Gendelman, R., Gallo, R. C. & Ensoli, B. Inflammatory cytokines induce endothelial cells to produce and release basic fibroblast growth factor and to promote Kaposi's sarcoma-like lesions in nude mice. *J. Immunol.* **158**, 1887–1894 (1997).
61. Aboulafia, D., Miles, S. A., Saks, S. R. & Mitsuyasu, R. T. Intravenous recombinant tumor necrosis factor in the treatment of AIDS-related Kaposi's sarcoma. *J. Acquir. Immune Defic. Syndr.* **2**, 54–58 (1989).
62. Krigel, R. L., Padavic-Shaller, K. A., Rudolph, A. R., Poiesz, B. J. & Comis, R. L. Exacerbation of epidemic Kaposi's sarcoma with a combination of interleukin-2 and β-interferon: results of a phase 2 study. *J. Biol. Response Mod.* **8**, 359–365 (1989).
63. Fagnoni, F. F. *et al.* Expansion of cytotoxic CD8$^+$ CD28$^-$ T cells in healthy ageing people, including centenarians. *Immunology* **88**, 501–507 (1996).
64. McNerlan, S. E., Rea, I. M. & Alexander, H. D. A whole blood method for measurement of intracellular TNF-α, IFN-γ and IL-2 expression in stimulated CD3$^+$ lymphocytes: differences between young and elderly subjects. *Exp. Gerontol.* **37**, 227–234 (2002).
65. Zanni, F. *et al.* Marked increase with age of type 1 cytokines within memory and effector/cytotoxic CD8$^+$ T cells in humans: a contribution to understand the relationship between inflammation and immunosenescence. *Exp. Gerontol.* **38**, 981–987 (2003).
66. Touloumi, G. *et al.* The role of immunosuppression and immune-activation in classic Kaposi's sarcoma. *Int. J. Cancer* **82**, 817–821 (1999).
67. Clerici, M. *et al.* Immune activation in africa is environmentally-driven and is associated with upregulation of CCR5. Italian-Ugandan AIDS Project. *AIDS* **14**, 2083–2092 (2000).
68. Kestens, L. *et al.* Endemic African Kaposi's sarcoma is not associated with immunodeficiency. *Int. J. Cancer* **36**, 49–54 (1985).
69. Rizzardini, G. *et al.* Immunological activation markers in the serum of African and European HIV-seropositive and seronegative individuals. *AIDS* **10**, 1535–1542 (1996).
70. Frances, C. *et al.* Outcome of kidney transplant recipients with previous human herpesvirus-8 infection. *Transplantation* **69**, 1776–1779 (2003).
71. Barete, S. *et al.* Clinical features and contribution of virological findings to the management of Kaposi sarcoma in organ-allograft recipients. *Arch. Dermatol.* **136**, 1452–1458 (2000).
72. Montagnino, G., Bencini, P. L., Tarantino, A., Caputo, R. & Ponticelli, C. Clinical features and course of Kaposi's sarcoma in kidney transplant patients: report of 13 cases. *Am. J. Nephrol.* **14**, 121–126 (1994).
73. Pelicci, P. G. *et al.* Multiple monoclonal B cell expansions and c-*myc* oncogene rearrangements in acquired immune deficiency syndrome-related lymphoproliferative disorders. Implications for lymphomagenesis. *J. Exp. Med.* **164**, 2049–2060 (2003).
74. Carbone, A. Emerging pathways in the development of AIDS-related lymphomas. *Lancet Oncol.* **4**, 22–29 (2003).
75. Lefevre, E. A., Krzysiek, R., Loret, E. P., Galanaud, P. & Richard, Y. Cutting edge: HIV-1 Tat protein differentially modulates the B cell response of naive, memory, and germinal center B cells. *J. Immunol.* **163**, 1119–1122 (1999).
76. Kundu, R. K. *et al.* Expression of the human immunodeficiency virus-Tat gene in lymphoid tissues of transgenic mice is associated with B-cell lymphoma. *Blood* **94**, 275–282 (1999).
77. Hazenberg, M. D. *et al.* T-cell division in human immunodeficiency virus (HIV)-1 infection is mainly due to immune activation: a longitudinal analysis in patients before and during highly active antiretroviral therapy (HAART). *Blood* **95**, 249–255 (2000).
78. Hazenberg, M. D. *et al.* Increased cell division but not thymic dysfunction rapidly affects the T-cell receptor excision circle content of the naive T cell population in HIV-1 infection. *Nature Med.* **9**, 1036–1042 (2000).
79. Morris, L. *et al.* HIV-1 antigen-specific and-nonspecific B cell responses are sensitive to combination antiretroviral therapy. *J. Exp. Med.* **188**, 233–245 (1998).
80. Zhang, Z. Q. *et al.* Kinetics of CD4$^+$ T cell repopulation of lymphoid tissues after treatment of HIV-1 infection. *Proc. Natl Acad. Sci. USA* **95**, 1154–1159 (1998).
81. Mohri, H. *et al.* Increased turnover of T lymphocytes in HIV-1 infection and its reduction by antiretroviral therapy. *J. Exp. Med.* **194**, 1277–1287 (2001).
82. Franco, J. M. *et al.* T-cell repopulation and thymic volume in HIV-1-infected adult patients after highly active antiretroviral therapy. *Blood* **99**, 3702–3706 (2002).
83. Pakker, N. G. *et al.* Biphasic kinetics of peripheral blood T cells after triple combination therapy in HIV-1 infection: a composite of redistribution and proliferation. *Nature Med.* **4**, 214–218 (1998).

84. Lebbe, C. *et al.* Clinical and biological impact of antiretroviral therapy with protease inhibitors on HIV-related Kaposi's sarcoma. *AIDS* **12**, F45–F49 (1998).
85. Gerard, L. *et al.* Improved survival in HIV-related Hodgkin's lymphoma since the introduction of highly active antiretroviral therapy. *AIDS* **17**, 81–87 (2003).
86. Antinori, A. *et al.* Better response to chemotherapy and prolonged survival in AIDS-related lymphomas responding to highly active antiretroviral therapy. *AIDS* **15**, 1483–1491 (2001).
87. Bower, M. *et al.* Highly active anti-retroviral therapy (HAART) prolongs time to treatment failure in Kaposi's sarcoma. *AIDS* **13**, 2105–2111 (1999).
Provides indirect evidence that HAART can be used as a maintenance therapy for patients with Kaposi's sarcoma because of effects unrelated to HIV suppression and immune reconstitution.
88. Hoffmann, C. *et al.* Response to highly active antiretroviral therapy strongly predicts outcome in patients with AIDS-related lymphoma. *AIDS* **17**, 1521–1529 (2003).
89. Holkova, B. *et al.* Effect of highly active antiretroviral therapy on survival in patients with AIDS-associated pulmonary Kaposi's sarcoma treated with chemotherapy. *J. Clin. Oncol.* **19**, 3848–3851 (2001).
90. Ahdieh-Grant, L. *et al.* Highly active antiretroviral therapy and cervical squamous intraepithelial lesions in human immunodeficiency virus-positive women. *J. Natl. Cancer Inst.* **96**, 1070–1076 (2004).
91. McGowan, J. P. & Shah, S. Long-term remission of AIDS-related primary central nervous system lymphoma associated with highly active antiretroviral therapy. *AIDS* **12**, 952–954 (1998).
92. Nasti, G. *et al.* AIDS-related Kaposi's Sarcoma: evaluation of potential new prognostic factors and assessment of the AIDS Clinical Trial Group Staging System in the Haart Era — the Italian Cooperative Group on AIDS and Tumors and the Italian Cohort of Patients Naive From Antiretrovirals. *J. Clin. Oncol.* **21**, 2876–2882 (2003).
93. Tam, H. K. *et al.* Effect of highly active antiretroviral therapy on survival among HIV-infected men with Kaposi sarcoma or non-Hodgkin lymphoma. *Int. J. Cancer* **98**, 916–922 (2002).
94. Wilkinson, J. *et al.* Identification of Kaposi's sarcoma-associated herpesvirus (KSHV)- specific cytotoxic T-lymphocyte epitopes and evaluation of reconstitution of KSHV-specific responses in human immunodeficiency virus type 1-Infected patients receiving highly active antiretroviral therapy. *J. Virol.* **76**, 2634–2640 (2002).
95. Carrieri, M. P. *et al.* Reduced incidence of Kaposi's sarcoma and of systemic non-hodgkin's lymphoma in HIV-infected individuals treated with highly active antiretroviral therapy. *Int. J. Cancer* **103**, 142–144 (2003).
96. Heard, I., Tassie, J. M., Kazatchkine, M. D. & Orth, G. Highly active antiretroviral therapy enhances regression of cervical intraepithelial neoplasia in HIV-seropositive women. *AIDS* **16**, 1799–1802 (2002).
97. Kostense, S. *et al.* Functional restoration of human immunodeficiency virus and Epstein–Barr virus-specific CD8$^+$ T cells during highly active antiretroviral therapy is associated with an increase in CD4$^+$ T cells. *Eur J. Immunol.* **32**, 1080–1089 (2002).
98. Grulich, A. E., Li, Y., Correll, P. K., Law, M. G. & Kaldor, J. M. Decreasing rates of Kaposi's sarcoma and non-Hodgkin's lymphoma in the era of potent combination anti-retroviral therapy. *AIDS* **15**, 629–633 (2001).
99. Sparano, J. A. *et al.* Effect of highly active antiretroviral therapy on the incidence of HIV-associated malignancies at an urban medical center. *J. Acquir. Immune Defic. Syndr.* **21**, S18–S22 (1999).
100. Gaidano, G., Capello, D. & Carbone, A. The molecular basis of acquired immunodeficiency syndrome-related lymphomagenesis. *Semin. Oncol.* **27**, 431–441 (2000).
101. Xu, J. *et al.* Analysis and significance of anti-latent membrane protein-1 antibodies in the sera of patients with EBV-associated diseases. *J. Immunol.* **164**, 2815–2822 (2000).
102. Duraiswamy, J. *et al. Ex vivo* analysis of T-cell responses to Epstein–Barr virus-encoded oncogene latent membrane protein 1 reveals highly conserved epitope sequences in virus isolates from diverse geographic regions. *J. Virol.* **77**, 7401–7410 (2003).
103. Cattelan, A. M. *et al.* Acquired immunodeficiency syndrome-related Kaposi's sarcoma regression after highly active antiretroviral therapy: biologic correlates of clinical outcome. *J. Natl Cancer Inst. Monogr.* **28**, 44–49 (2001).
104. Chotmongkol, V. & Pesee, M. AIDS-related primary central nervous system lymphoma: prolonged remission associated with highly active antiretroviral therapy. *J. Med. Assoc. Thai.* **85**, 634–637 (2002).
105. Dupont, C. *et al.* Long-term efficacy on Kaposi's sarcoma of highly active antiretroviral therapy in a cohort of HIV-positive patients. CISIH 92. Centre d'information et de soins de l'immunodeficience humaine. *AIDS* **14**, 987–993 (2000).
106. Navarro, J. T., Ribera, J. M., Oriol, A., Milla, F. & Feliu, E. Improved outcome of AIDS-related lymphoma in patients with virologic response to highly active antiretroviral therapy. *J. Acquir. Immune Defic. Syndr.* **32**, 347–348 (2003).
107. Nunez, M., Machuca, A., Soriano, V., Podzamczer, D. & Gonzalez-Lahoz, J. Clearance of human herpesvirus type 8 viraemia in HIV-1-positive patients with Kaposi's sarcoma treated with liposomal doxorubicin. Caelyx/KS Spanish Study Group. *AIDS* **14**, 913–919 (2000).
108. Pellet, C. *et al.* Virologic and immunologic parameters that predict clinical response of AIDS-associated Kaposi's sarcoma to highly active antiretroviral therapy. *J. Invest. Dermatol.* **117**, 858–863 (2001).
109. Conant, M. A., Opp, K. M., Poretz, D. & Mills, R. G. Reduction of Kaposi's sarcoma lesions following treatment of AIDS with ritonovir. *AIDS* **11**, 1300–1301 (1997).
110. Hocqueloux, L., Agbalika, F., Oksenhendler, E. & Molina, J. M. Long-term remission of an AIDS-related primary effusion lymphoma with antiviral therapy. *AIDS* **15**, 280–282 (2001).
111. Murphy, M., Armstrong, D., Sepkowitz, K. A., Ahkami, R. N. & Myskowski, P. L. Regression of AIDS-related Kaposi's sarcoma following treatment with an HIV-1 protease inhibitor. *AIDS* **11**, 261–262 (1997).
112. Paparizos, V. A., Kyriakis, K. P., Papastamopoulos, V., Hadjivassiliou, M. & Stavrianeas, N. G. Response of AIDS-associated Kaposi sarcoma to highly active antiretroviral therapy alone. *J. Acquir. Immune. Defic. Syndr.* **30**, 257–258 (2002).
113. Parra, R. *et al.* Regression of invasive AIDS-related Kaposi's sarcoma following antiretroviral therapy. *Clin. Infect. Dis.* **26**, 218–219 (1998).
114. Robinson, W. R., Hamilton, C. A., Michaels, S. H. & Kissinger, P. Effect of excisional therapy and highly active antiretroviral therapy on cervical intraepithelial neoplasia in women infected with human immunodeficiency virus. *Am. J. Obstet. Gynecol.* **184**, 538–543 (2001).
115. Robinson, W. R. & Freeman, D. Improved outcome of cervical neoplasia in HIV-infected women in the era of highly active antiretroviral therapy. *AIDS Patient Care STDS* **16**, 61–65 (2002).
116. Sarkodee-Adoo, C. *et al.* Regression and clonally distinct recurrence of human immunodeficiency virus related Burkitt-like lymphoma during antiretroviral therapy. *Leuk. Lymphoma* **42**, 1125–1131 (2001).
117. Uberti-Foppa, C. *et al.* Long-term effect of highly active antiretroviral therapy on cervical lesions in HIV-positive women. *AIDS* **17**, 2136–2138 (2003).
118. Boivin, G. *et al.* Evaluation of the human herpesvirus 8 DNA load in blood and Kaposi's sarcoma skin lesions from AIDS patients on highly active antiretroviral therapy. *AIDS* **14**, 1907–1910 (2000).
119. Benfield, T. L., Kirk, O., Elbrond, B. & Pedersen, C. Complete histological regression of Kaposi's sarcoma following treatment with protease inhibitors despite persistence of HHV-8 in lesions. *Scand. J. Infect. Dis.* **30**, 613–615 (1998).
120. De Milito A. *et al.* Antiretroviral therapy with protease inhibitors in human immunodeficiency virus type 1- and human herpesvirus 8-coinfected patients. *J. Med. Virol.* **57**, 140–144 (1999).
121. Torre-Cisneros, J. *et al.* Patterns of lymphotropic herpesvirus viraemia in HIV-infected patients with Kaposi's sarcoma treated with highly active antiretroviral therapy and liposomal daunorubicin. *AIDS* **14**, 2215–2217 (2000).
122. Bourboulia, D. *et al.* Short- and long-term effects of highly active antiretroviral therapy on Kaposi sarcoma-associated herpesvirus immune responses and viraemia. *AIDS* **18**, 485–493 (2004).
123. Cuzick, J., Terry, G., Ho, L., Hollingworth, T. & Anderson, M. Human papillomavirus type 16 in cervical smears as predictor of high-grade cervical intraepithelial neoplasia. *Lancet* **339**, 959–960 (1992).
124. Remmink, A. J. *et al.* The presence of persistent high-risk HPV genotypes in dysplastic cervical lesions is associated with progressive disease: natural history up to 36 months. *Int. J. Cancer* **61**, 306–311 (1995).
125. Stebbing, J. *et al.* The efficacy of ritonavir in the prevention of AIDS-related Kaposi's sarcoma. *Int. J. Cancer* **108**, 631–633 (2004).
126. Gill, J. *et al.* Prospective study of the effects of antiretroviral therapy on Kaposi sarcoma: associated herpesvirus infection in patients with and without Kaposi sarcoma. *J. Acquir. Immune. Defic. Syndr.* **31**, 384–390 (2002).
127. Ridolfo, A. L. *et al.* Is switching protease inhibitor-based effective antiretroviral therapy safe in patients with AIDS-associated Kaposi's sarcoma? *AIDS* **18**, 1224–1226 (2004).
128. Bani-Sadr, F., Fournier, S. & Molina, J. M. Relapse of Kaposi's sarcoma in HIV-infected patients switching from a protease inhibitor to a non-nucleoside reverse transcriptase inhibitor-based highly active antiretroviral therapy regimen. *AIDS* **17**, 1580–1581 (2003).
129. Gaedicke, S. *et al.* Antitumor effect of the human immunodeficiency virus protease inhibitor ritonavir: induction of tumor-cell apoptosis associated with perturbation of proteasomal proteolysis. *Cancer Res.* **62**, 6901–6908 (2002).
Showed that HIV-PIs can modulate proteasome activity to increase the half-life of WAF1 with no effects on proteasome-mediated degradation of the protein bulk.
130. Pati, S. *et al.* Antitumorigenic effects of HIV protease inhibitor ritonavir: inhibition of Kaposi sarcoma. *Blood* **99**, 3771–3779 (2002).
131. Ensoli, B. *et al.* AIDS-Kaposi's sarcoma-derived cells express cytokines with autocrine and paracrine growth effects. *Science* **243**, 223–226 (1989).
132. Ensoli, B. *et al.* Block of AIDS-Kaposi's sarcoma (KS) cell growth, angiogenesis, and lesion formation in nude mice by antisense oligonucleotide targeting basic fibroblast growth factor. A novel strategy for the therapy of KS. *J. Clin. Invest.* **94**, 1736–1746 (1994).
133. Weichold, F. F. *et al.* HIV-1 protease inhibitor ritonavir modulates susceptibility to apoptosis of uninfected T cells. *J. Hum. Virol.* **2**, 261–269 (1999).
134. Tovo, P. A. Highly active antiretroviral therapy inhibits cytokine production in HIV-uninfected subjects. *AIDS* **14**, 743–744 (2000).
Showed that PI–HAART inhibited, in two HIV-uninfected individuals, the production of inflammatory cytokines that are known to lead to the development of Kaposi's sarcoma.
135. Bissell, M. J. & Radisky, D. Putting tumours in context. *Nature Rev. Cancer* **1**, 46–54 (2001).
136. Chavan, S., Kodoth, S., Pahwa, R. & Pahwa, S. The HIV protease inhibitor Indinavir inhibits cell-cycle progression *in vitro* in lymphocytes of HIV-infected and uninfected individuals. *Blood* **98**, 383–389 (2001).
137. Lu, W. & Andrieu, J. M. HIV protease inhibitors restore impaired T-cell proliferative response *in vivo* and *in vitro*: a viral-suppression-independent mechanism. *Blood* **96**, 250–258 (2000).
138. Sloand, E. M. *et al.* Human immunodeficiency virus type 1 protease inhibitor modulates activation of peripheral blood CD4$^+$ T cells and decreases their susceptibility to apoptosis *in vitro* and *in vivo*. *Blood* **94**, 1021–1027 (1999).
139. Gruber, A., Wheat, J. C., Kuhen, K. L., Looney, D. J. & Wong-Staal, F. Differential effects of HIV-1 protease inhibitors on dendritic cell immunophenotype and function. *J. Biol. Chem.* **276**, 47840–47843 (2001).
Reported that HIV-PIs can affect maturation and function of professional antigen-presenting cells, with implications for tumour immunity.
140. Kelleher, A. D. *et al.* Effects of retroviral protease inhibitors on proteasome function and processing of HIV-derived MHC class I-restricted cytotoxic T lymphocyte epitopes. *AIDS Res. Hum. Retroviruses* **17**, 1063–1066 (2001).
141. Sloand, E. M. *et al.* Protease inhibitors stimulate hematopoiesis and decrease apoptosis and ICE expression in CD34$^+$ cells. *Blood* **96**, 2735–2739 (2000).
142. Zhong, D. S. *et al.* HIV protease inhibitor ritonavir induces cytotoxicity of human endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* **22**, 1560–1566 (2002).
143. Schmidtke, G. *et al.* How an inhibitor of the HIV-I protease modulates proteasome activity. *J. Biol. Chem.* **274**, 35734–35740 (1999).
144. Hosseini, H. *et al.* Protection against experimental autoimmune encephalomyelitis by a proteasome modulator. *J. Neuroimmunol.* **118**, 233–244 (2001).
145. Piccinini, M. *et al.* The human 26S proteasome is a target of antiretroviral agents. *AIDS* **16**, 693–700 (2002).
146. Goldberg, A. L. & Rock, K. Not just research tools—proteasome inhibitors offer therapeutic promise. *Nature Med.* **8**, 338–340 (2002).
147. Lenz, H. J. Clinical update: proteasome inhibitors in solid tumors. *Cancer Treat. Rev.* **29**, 41–48 (2003).
148. Palombella, V. J., Rando, O. J., Goldberg, A. L. & Maniatis, T. The ubiquitin-proteasome pathway is required for processing the NF-κB1 precursor protein and the activation of NF-κB. *Cell* **75**, 773–785 (1994).
149. Russo, S. M. *et al.* Enhancement of radiosensitivity by proteasome inhibition: implications for a role of NF-κB. *Int. J. Radiat. Oncol. Biol. Phys.* **50**, 183–193 (2001).
150. Schmidtke, G., Emch, S., Groettrup, M. & Holzhutter, H. G. Evidence for the existence of a non-catalytic modifier site of peptide hydrolysis by the 20S proteasome. *J. Biol. Chem.* **275**, 22056–22063 (2000).
151. Adams, J. Proteasome inhibitors as new anticancer drugs. *Curr. Opin. Oncol* **14**, 628–634 (2002).

152. Gaczynska, M., Rock, K. L. & Goldberg, A. L. γ-interferon and expression of MHC genes regulate peptide hydrolysis by proteasomes. *Nature* **365**, 264–267 (1993).
153. Liang, J. S. *et al.* HIV protease inhibitors protect apolipoprotein B from degradation by the proteasome: a potential mechanism for protease inhibitor-induced hyperlipidemia 126. *Nature Med.* **7**, 1327–1331 (2001).
A paper providing compelling evidence that the cell proteasome in targeted by HIV-PIs in treated patients.
154. Ensoli, B. Employment of human immunodeficiency virus (HIV) protease inhibitors (HIV-PI) for utilizing them as drugs or realizing new anti-angiogenic, anti-tumor, anti-edemic, anti-inflammatory drugs, for the treatment of Kaposi's sarcoma, tumors and angioproliferative, inflammatory and autoimmune diseases in HIV infected or non infected subject. RM2001A000210 (2001).
155. Bafetti, L. M., Young, T. N., Itoh, Y. & Stack, M. S. Intact vitronectin induces matrix metalloproteinase-2 and tissue inhibitor of metalloproteinases-2 expression and enhanced cellular invasion by melanoma cells. *J. Biol. Chem.* **273**, 143–149 (1998).
156. Kheradmand, F., Werner, E., Tremble, P., Symons, M. & Werb, Z. Role of Rac1 and oxygen radicals in collagenase-1 expression induced by cell shape change. *Science* **280**, 898–902 (1998).
157. Vaccher, E., di, G. G., Nasti, G., Juzbasic, S. & Tirelli, U. HAART is effective as anti-Kaposi's sarcoma therapy only after remission has been induced by chemotherapy. *J. Acquir. Immune Defic. Syndr.* **22**, 407–408 (1999).
158. Lopez, D., Gil-Torregrosa, B. C., Bergmann, C. & Del, V. M. Sequential cleavage by metallopeptidases and proteasomes is involved in processing HIV-1 ENV epitope for endogenous MHC class I antigen presentation. *J. Immunol.* **164**, 5070–5077 (2000).
159. Kobayashi, Y., Matsumoto, M., Kotani, M. & Makino, T. Possible involvement of matrix metalloproteinase-9 in Langerhans cell migration and maturation. *J. Immunol.* **163**, 5989–5993 (1999).
160. Gearing, A. J. *et al.* Processing of tumour necrosis factor-α precursor by metalloproteinases. *Nature* **370**, 555–557 (1994).
161. Ito, A. *et al.* Degradation of interleukin 1β by matrix metalloproteinases. *J. Biol. Chem.* **271**, 14657–14660 (1996).
162. McQuibban, G. A. *et al.* Inflammation dampened by gelatinase A cleavage of monocyte chemoattractant protein-3. *Science* **289**, 1202–1206 (2000).
163. Schonbeck, U., Mach, F. & Libby, P. Generation of biologically active IL-1β by matrix metalloproteinases: a novel caspase-1-independent pathway of IL-1β processing. *J. Immunol.* **161**, 3340–3346 (1998).
164. Van den Steen, P. E., Proost, P., Wuyts, A., Van, D. J. & Opdenakker, G. Neutrophil gelatinase B potentiates interleukin-8 tenfold by aminoterminal processing, whereas it degrades CTAP-III, PF-4, and GRO-α and leaves RANTES and MCP-2 intact. *Blood* **96**, 2673–2681 (2000).
165. Gorelik, L. & Flavell, R. A. Immune-mediated eradication of tumors through the blockade of transforming growth factor-β signaling in T cells. *Nature Med.* **7**, 1118–1122 (2001).
166. Yu, Q. & Stamenkovic, I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-β and promotes tumor invasion and angiogenesis. *Genes Dev.* **14**, 163–176 (2000).
167. Sheu, B. C. *et al.* A novel role of metalloproteinase in cancer-mediated immunosuppression. *Cancer Res.* **61**, 237–242 (2001).
168. Martignetti, J. A. *et al.* Mutation of the matrix metalloproteinase 2 gene (MMP2) causes a multicentric osteolysis and arthritis syndrome. *Nature Genet.* **28**, 261–265 (2001).
Shows that genetic loss of MMP2 function results in bone disorders. These defects also occur in HAART-treated patients.
169. Mora, S. *et al.* Bone mineral loss through increased bone turnover in HIV-infected children treated with highly active antiretroviral therapy. *AIDS* **15**, 1823–1829 (2001).
170. Tebas, P. *et al.* Accelerated bone mineral loss in HIV-infected patients receiving potent antiretroviral therapy. *AIDS* **14**, F63–F67 (2000).
171. Ferrara, N., Hillan, K. J., Gerber, H. P. & Novotny, W. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. *Nature Rev. Drug Discov.* **3**, 391–400 (2004).
172. Coussens, L. M. & Werb, Z. Inflammation and cancer. *Nature* **420**, 860–867 (2002).
173. Arun, B. & Goss, P. The role of COX-2 inhibition in breast cancer treatment and prevention. *Semin. Oncol.* **31**, 22–29 (2004).
174. Tirelli, U., Bernardi, D., Spina, M. & Vaccher, E. AIDS-related tumors: integrating antiviral and anticancer therapy. *Crit. Rev. Oncol. Hematol.* **41**, 299–315 (2002).
175. Ratner, L. *et al.* Chemotherapy for human immunodeficiency virus-associated non-Hodgkin's lymphoma in combination with highly active antiretroviral therapy. *J. Clin. Oncol.* **19**, 2171–2178 (2001).
176. Nannan, P. V. *et al.* Paclitaxel in the treatment of human immunodeficiency virus 1-associated Kaposi's sarcoma—drug-drug interactions with protease inhibitors and a nonnucleoside reverse transcriptase inhibitor: a case report study. *Cancer Chemother. Pharmacol.* **43**, 516–519 (1999).
177. Vaccher, E. *et al.* Concomitant cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy plus highly active antiretroviral therapy in patients with human immunodeficiency virus-related, non-Hodgkin lymphoma. *Cancer* **91**, 155–163 (2001).
178. Spina, M. *et al.* Stanford V regimen and concomitant HAART in 59 patients with Hodgkin disease and HIV infection. *Blood* **100**, 1984–1988 (2002).
179. Mastroianni, C. M. *et al. Ex vivo* and *in vitro* effect of human immunodeficiency virus protease inhibitors on neutrophil apoptosis. *J. Infect. Dis.* **182**, 1536–1539 (2000).
180. Isgro, A. *et al.* Recovery of hematopoietic activity in bone marrow from human immunodeficiency virus type 1-infected patients during highly active antiretroviral therapy. *AIDS Res. Hum. Retroviruses* **16**, 1471–1479 (2000).
181. Adams, G. B., Pym, A. S., Poznansky, M. C., McClure, M. O. & Weber, J. N. The *in vivo* effects of combination antiretroviral drug therapy on peripheral blood $CD34^+$ cell colony-forming units from HIV type 1-infected patients. *AIDS Res. Hum. Retroviruses* **15**, 551–559 (1999).
182. Olson, D. P., Scadden, D. T., D'Aquila, R. T. & De Pasquale, M. P. The protease inhibitor ritonavir inhibits the functional activity of the multidrug resistance related-protein 1 (MRP-1). *AIDS* **16**, 1743–1747 (2002).
183. Ikebe, T. *et al.* Involvement of proteasomes in migration and matrix metalloproteinase-9 production of oral squamous cell carcinoma. *Int. J. Cancer* **77**, 578–585 (1998).
184. Li, J. *et al.* PR39, a peptide regulator of angiogenesis. *Nature Med.* **6**, 49–55 (2000).
185. Mezquita, J., Mezquita, B., Pau, M. & Mezquita, C. Down-regulation of *Flt-1* gene expression by the proteasome inhibitor MG262. *J. Cell. Biochem.* **89**, 1138–1147 (2003).
186. Oikawa, T. *et al.* The proteasome is involved in angiogenesis. *Biochem. Biophys. Res Commun.* **246**, 243–248 (1998).
187. Cocchi, F. *et al.* The V3 domain of the HIV-1 gp120 envelope glycoprotein is critical for chemokine-mediated blockade of infection. *Nature Med.* **2**, 1244–1247 (1996).
188. Douek, D. C. *et al.* Evidence for increased T cell turnover and decreased thymic output in HIV infection. *J. Immunol.* **167**, 6663–6668 (2001).
189. Gallagher, B., Wang, Z., Schymura, M. J., Kahn, A. & Fordyce, E. J. Cancer incidence in New York State acquired immunodeficiency syndrome patients. *Am. J. Epidemiol.* **154**, 544–556 (2001).
190. Bower, M. *et al.* HIV-related lung cancer in the era of highly active antiretroviral therapy. *AIDS* **17**, 371–375 (2003).
191. Powles, T. *et al.* Multicenter study of human immunodeficiency virus-related germ cell tumors. *J. Clin. Oncol.* **21**, 1922–1927 (2003).
192. Herida, M. *et al.* Incidence of non-AIDS-defining cancers before and during the highly active antiretroviral therapy era in a cohort of human immunodeficiency virus-infected patients. *J. Clin. Oncol.* **21**, 3447–3453 (2003).
193. Powles, T. *et al.* Outcome of patients with HIV-related germ cell tumours: a case-control study. *Br. J. Cancer* **90**, 1526–1530 (2004).
194. Sgadari, C., Monini, P., Barillari, G. & Ensoli, B. Use of HIV protease inhibitors to block Kaposi's sarcoma and tumour growth. *Lancet Oncol.* **4**, 537–547 (2003).
195. Phenix, B. N., Lum, J. J., Nie, Z., Sanchez-Dardon, J. & Badley, A. D. Antiapoptotic mechanism of HIV protease inhibitors: preventing mitochondrial transmembrane potential loss. *Blood* **98**, 1078–1085 (2001).

Acknowledgments

We are grateful to H. Kleinman for helpful discussion and to Emanuela Vaccher for discussion on the potential clinical use of HIV-PIs in patients with cancer. We thank A. Carinci, S. Ceccarelli and F. M. Regini for editorial assistance. The work by the authors was supported by grants from Associazione Italiana per la Ricerca sul Cancro (AIRC) and from the Italian Ministry of Health (Ricerca Finalizzata) to B. E.

Competing interests statement

The authors declare no competing financial interests.

Online links

DATABASES

The following terms in this article are linked online to:

Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
bFGF | COX2 | ICAM1 | IFNγ | IL-1β | IL-2 | IL-2 receptor-α | IL-6 | IL-8 | MMP2 | MMP3 | MMP7 | monocyte chemoattractant protein 3 | MT1-MMP | selectin E | TNFα | TRAIL | transforming growth factor-β | VCAM1 | VEGF | WAF1

National Cancer Institute: http://cancer.gov/
cervical cancer | non-Hodgkin's lymphomas

OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=omim
Kaposi's sarcoma

FURTHER INFORMATION

European Organisation for Research and Treatment of Cancer: www.eortc.be/
GenBank: www.ncbi.nih.gov/Genbank
HIV/AIDS Treatment Information Service: www.hivatis.org
Medscape Druginfo: www.medscape.com/druginfo
Southwest Oncology Group: http://swog.org/

Access to this interactive links box is free online.