

Anti-HIV drugs for cancer therapeutics: back to the future?

Warren A Chow, Chunling Jiang, Min Guan

The use of anti-HIV drugs as cancer treatments is not new. Azidothymidine was studied as an antineoplastic in the 1990s, but despite promising *in vitro* data, clinical trials showed little antitumour activity. HIV protease inhibitors were developed in the early 1990s, and their subsequent incorporation into highly active antiretroviral therapy (HAART) has profoundly changed the natural history of HIV infection. The potential antitumour properties of these drugs have been investigated because of their success in treating HIV-related Kaposi's sarcoma. HAART's effects on Kaposi's sarcoma did not always correlate with immune reconstitution, and activity against other solid and haematological malignancies has been established. Inhibition of tumour-cell invasion and angiogenesis were properties first ascribed to inhibition of HIV protease; however, they have pleiotropic antitumour effects, including inhibition of inflammatory cytokine production, proteasome activity, cell proliferation and survival, and induction of apoptosis. HIV protease inhibitors are thus a new class of anticancer drugs with multiple effects, and other anti-HIV drugs might hold similar promise.

Introduction

Sir James W Black—the pharmacologist and 1988 Nobel laureate in medicine—famously said the “most fruitful basis of the discovery of a new drug is to start with an old drug” (figure 1). The development of new treatments for cancer is both time-consuming and enormously costly. An analysis of 68 approved drugs estimated that it takes an average of 15 years and US\$802 million (in US dollars at prices and exchange rates in the year 2000) to successfully develop a new drug.¹ If postmarketing safety costs are included, total costs rise to nearly \$900 million (figure 2).² Consequently, the repositioning of old drugs for alternative purposes, such as cancer treatment, is timely. Several successful examples of repositioned drugs in the treatment of cancer exist. Retinoic acid, originally developed for acne is approved for the treatment of acute promyelocytic leukaemia. Arsenic trioxide, known as the oldest drug in the world, is also approved for the treatment of the same disorder. Perhaps the most successfully repositioned drug is thalidomide: originally developed as a sedative for pregnant women (with disastrous birth-defect results), it is now approved for therapy of multiple myeloma. These successes prove the feasibility of developing existing, approved drugs for cancer therapeutics. Anti-HIV drugs target multiple pathways of the HIV life cycle, and provide a large source of potential anticancer drugs.

Nucleoside inhibitors of reverse transcriptase

Azidothymidine

Jerome Horwitz of Barbara Ann Karmanos Cancer Institute and Wayne State University School of Medicine synthesised zidovudine (azidothymidine), the first antiviral drug used to treat HIV, in 1964. The compound was first synthesised as a treatment for cancer, but had little anticancer activity and unacceptable toxicity. No further research with zidovudine was pursued until 1984, when Marty St Clair discovered that the drug inhibited growth of HIV *in vitro*.³ Within months, Samuel Broder and colleagues at the US National Cancer Institute, confirmed these results, which rapidly led to clinical

trials showing that zidovudine could increase CD4 cell counts and prolong survival of people infected with HIV.^{4,5} The Food and Drug Administration (FDA) approved zidovudine for use against HIV infection on March 20, 1987, and as a preventive treatment in 1990.

Interest in zidovudine as an anticancer drug was renewed in 1989 when researchers found that the drug could restore sensitivity to cisplatin in resistant cells *in vitro*.⁶ Zidovudine cytotoxicity is related to its incorporation into tumour cell DNA by DNA polymerase; however, this reaction is not favoured in human beings under normal conditions, and therefore has little antitumour activity as a single agent.^{7,8} *In-vitro* and *in-vivo* studies showed that inhibition of *de novo* synthesis of deoxythymidine monophosphate (dTMP) with fluorouracil and methotrexate resulted in increased zidovudine cytotoxicity in

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Beckman Research Institute of the City of Hope, Division of Medical Oncology, Department of Clinical and Molecular Pharmacology, Duarte, CA USA (W A Chow MD, C Jiang PhD, M Guan PhD)

Correspondence to: Dr Warren A Chow, Beckman Research Institute of the City of Hope, Division of Medical Oncology, Department of Clinical and Molecular Pharmacology, 1500 E Duarte Rd, Duarte, CA 91010, USA
wchow@coh.org



Figure 1: Reclassifying an approved drug for cancer therapeutics

In December, 1995, encouraging clinical trial results led the US Food and Drug Administration to quickly approve a new drug to be used in combination with others. That drug was called saquinavir, the world's first protease inhibitor.

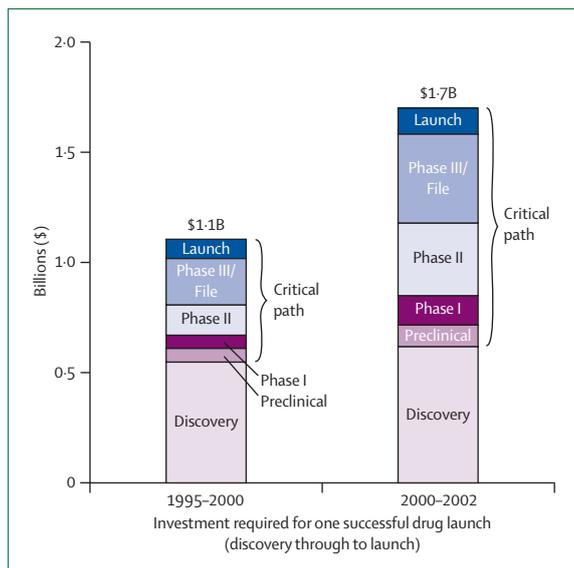


Figure 2: Growing cost of successful drug development
The cost of successful drug development increased 55% from 1995–2000 to 2000–02. Most of the rising costs are attributable to clinical trials. Reproduced with permission from reference 2.

various cell lines and more zidovudine-induced DNA strand breaks.^{7,9,10} In this circumstance, thymidine salvage, which is mediated by thymidine kinase, becomes the main source of dTMP for support of DNA synthesis. Thymidine kinase also phosphorylates zidovudine, which, as a triphosphate, competes with dTTP in DNA synthesis.⁹ These in-vitro results suggested synergistic activity between zidovudine and chemotherapy.

Several phase I trials of zidovudine in combination with chemotherapy with fluorouracil, methotrexate, and cisplatin have been reported.^{8,11–13} The combination of intravenous zidovudine with fluorouracil was originally reported in a phase I/II study in previously untreated patients with metastatic colorectal cancer.⁸ The initial zidovudine dose was 0.5 g/m² with fluorouracil 500 mg/m² intravenously once a week with intravenous calcium folinate (leucovorin). The dose-limiting toxicity for zidovudine was diarrhoea at 10 g/m². The objective response rate was an impressive 44%. Pharmacokinetic analysis showed that the peak plasma concentration of zidovudine was 995.6 µmol/L. A subsequent phase I trial of continuous-infusion fluorouracil, calcium folinate, and intravenous zidovudine was reported.¹¹ The dose of azidothymidine reached 20 g/m², and the greatest tolerated dose was not achieved. Dose-dependent DNA strand breaks were observed in peripheral blood cells in these patients, with peak plasma zidovudine concentrations reaching 133 µmol/L for high-dose zidovudine. No patients in this heavily pretreated population had objective responses, which was not surprising given that only much higher concentrations (500–1000 µmol/L) were effective in mouse models.¹¹ A phase II trial of oral zidovudine (2 g/m² days 1–3, 4 g/m² days 8–10, and

6 g/m² days 15–17) and weekly intravenous methotrexate 1 g/m² plus calcium folinate rescue was reported in patients with HIV-related non-Hodgkin lymphoma.¹² The response rate was 77% with complete response rate of 46% with median duration of complete remission of 12.8 months. Finally, our group did a phase I study¹³ of zidovudine (400 mg/m² per day to 14.4 g/m² per day) given as a continuous infusion on days 1–3 and days 14–16 of a 28-day cycle and cisplatin 30–60 mg/m² given on day 2 of each zidovudine infusion. Dose-limiting toxicity was myelosuppression. The mean steady-state concentration of zidovudine at the highest tolerated dose was 44 µmol/L. Stable disease for a median of four cycles was achieved in 34% in this heavily pretreated population of patients. These results show the feasibility of combining zidovudine with chemotherapy. Assessment of the contribution of the antitumour activity of zidovudine to these regimens is difficult, because none of the trials had a chemotherapy-only arm (or a placebo-control) for comparison.

In combination with the cytokine, interferon alfa, zidovudine induces apoptosis in vitro in human herpesvirus virus 8 (HHV8) associated primary effusion lymphomas, a unique subtype of non-Hodgkin lymphoma nearly always associated with HIV infection.^{14,15} Either agent alone had little activity; however, the combination showed synergistic activity. Zidovudine induced upregulation of either Fas or tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) and inhibited activation of nuclear factor-κB (NFκB) accounting for its activity. Furthermore, a patient with primary effusion lymphoma had complete remission after treatment with zidovudine and interferon alfa.¹⁶ Despite its unique activity in HIV-associated primary effusion lymphoma, zidovudine has little activity against HIV-related Kaposi's sarcoma. Only two of 22 individuals with HIV with Kaposi's sarcoma in a phase II trial of zidovudine showed evidence of response lasting only 2 months or 4 months.¹⁷

Cidofovir

Both endemic and HIV-related Kaposi's sarcoma are invariably associated with infection with HHV8.¹⁸ An antiviral, nucleoside monophosphate analogue, cidofovir, inhibits growth of Kaposi's sarcoma in vitro and nasopharyngeal carcinoma caused by Epstein-Barr virus in nude mice.^{19,20} Complete remission has been reported for both HIV-related and HIV-negative primary effusion lymphoma after intracavitary administration of cidofovir.^{21,22} This drug inhibits development of non-oncogenic virus-associated haemangiosarcoma in mice.²³ Cidofovir was incorporated into tumoral DNA through DNA polymerase, but required a dose 25 times higher than that required for protective antiviral activity in mice infected with herpesvirus mice, suggesting the restricted role of this drug in non-oncogenic virus-associated tumours.

Ganciclovir

The risk of Kaposi's sarcoma was significantly lower in people infected with HIV who received oral or intravenous ganciclovir (9-[1,3-dihydroxy-2-propoxy-methyl]guanine) for cytomegalovirus retinitis.²⁴ Like cidofovir, ganciclovir is active, although much less potent, in vitro against HHV8.^{19,25} Consequently, ganciclovir's failure to inhibit growth of primary effusion lymphoma in culture or in murine xenografts is not surprising.²⁶

Non-nucleoside inhibitors of reverse transcriptase

Undifferentiated cells and embryos express large amounts of endogenous reverse transcriptase of retroviral origin, which enables them to retrotranspose autonomously.²⁷ Nevirapine and efavirenz reduced proliferation, induced morphological differentiation, and reprogrammed gene expression in transformed melanoma and prostate carcinoma cells without inducing apoptosis. Further, efavirenz inhibited the growth of xenografted melanoma, prostate, colon, and small-cell lung carcinoma tumours in nude mice.²⁷ On the basis of these results, the researchers postulated that endogenous reverse transcriptase acts as an epigenetic regulator of cell differentiation and proliferation.²⁷

The use of nevirapine and efavirenz were consequently investigated as treatments for anaplastic thyroid cancer, an aggressive form in which specific thyroid-cell functions are typically lost, such as production of thyroglobulin, ability to take up iodine, and expression of the thyroid-stimulating-hormone (TSH) receptor.²⁸ Treated cells had a differentiated phenotype and re-established expression of thyroglobulin and the TSH receptor, and uptake of iodine.²⁸ A 76-year-old female who presented with metastatic thyroid papillary carcinoma provided proof of concept.²⁹ The patient had thyroidectomy followed by three radioiodine treatments. Restaging showed that distant metastases did not take up radioiodine, and a biopsy of a lymph-node metastasis showed progressive dedifferentiation of the initial thyroid papillary carcinoma. Thyroglobulin expression was undetectable. She received treatment with nevirapine 200 mg twice daily. 2 months after starting nevirapine, thyroglobulin expression became detectable, radioiodine uptake was re-established, and a biopsy of the lymph-node metastasis showed reduced nuclear pleomorphism.

HIV-protease inhibitors

Development of HIV-protease inhibitors in the early 1990s followed the characterisation of the crystal structure of HIV protease in 1989.³⁰ Inhibitors of HIV protease are peptidomimetics that generally contain a synthetic analogue of the peptide bond between phenylalanine and proline at positions 167 and 168 of the gag-pol polyprotein, which is the target of the HIV

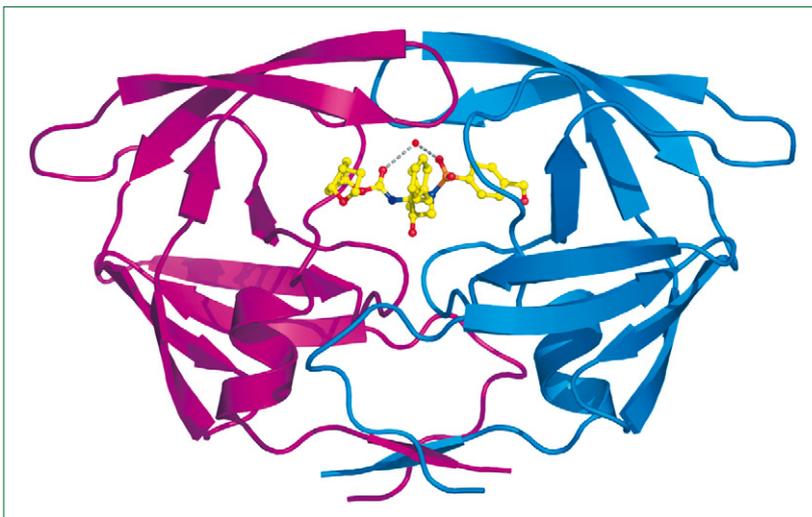


Figure 3: Structure of the HIV-1 protease bound to a protease inhibitor

The protease dimer with an inhibitor molecule (yellow) bound in the active site is shown. Reproduced with permission from Prof J E Wedekind, adapted from reference 31.

aspartyl protease (figure 3).^{32,33} The first inhibitor of HIV protease developed that received FDA approval was saquinavir (figure 1), followed by ritonavir, indinavir, nelfinavir, and amprenavir. Drugs approved more recently include lopinavir (in combination with ritonavir), atazanavir, fosamprenavir (a prodrug of amprenavir), tipranavir, and darunavir (figure 4). The combination of a protease inhibitor and two nucleoside or non-nucleoside reverse transcriptase inhibitors as highly active antiretroviral therapy (HAART) led to remarkable suppression of HIV replication, leading to restoration of immunity and reduction of HIV-related morbidity and mortality.³⁴

Early in the HAART era, investigators reported regression of Kaposi's sarcoma and Kaposi's sarcoma-free survival in individuals on HAART.^{35,36} Kaposi's sarcoma is an angioproliferative disease characterised by angiogenesis, endothelial spindle-cell growth (Kaposi's sarcoma cells), inflammatory-cell infiltration, and oedema, which become progressively infected by human herpesvirus 8.³⁷ Subsequently, large prospective cohort trials of people with HIV on HAART reported significant reductions in the incidence of Kaposi's sarcoma and later, HIV-related non-Hodgkin lymphoma.^{38,39} At first thought to be a consequence of effective anti-HIV treatment and immune reconstitution, the effects of HAART on Kaposi's sarcoma control did not always correlate well with HIV load or CD4 cell counts. Progression of Kaposi's sarcoma while on HAART was not necessarily associated with virological failure in a fifth of the patients in one study.³⁶ In fact, regression was occasionally so rapid, that it preceded the reduction in HIV titres.³⁵ These were the earliest clinical indications that protease-inhibitor antitumour activity had a non-immune-mediated mechanism. As well as

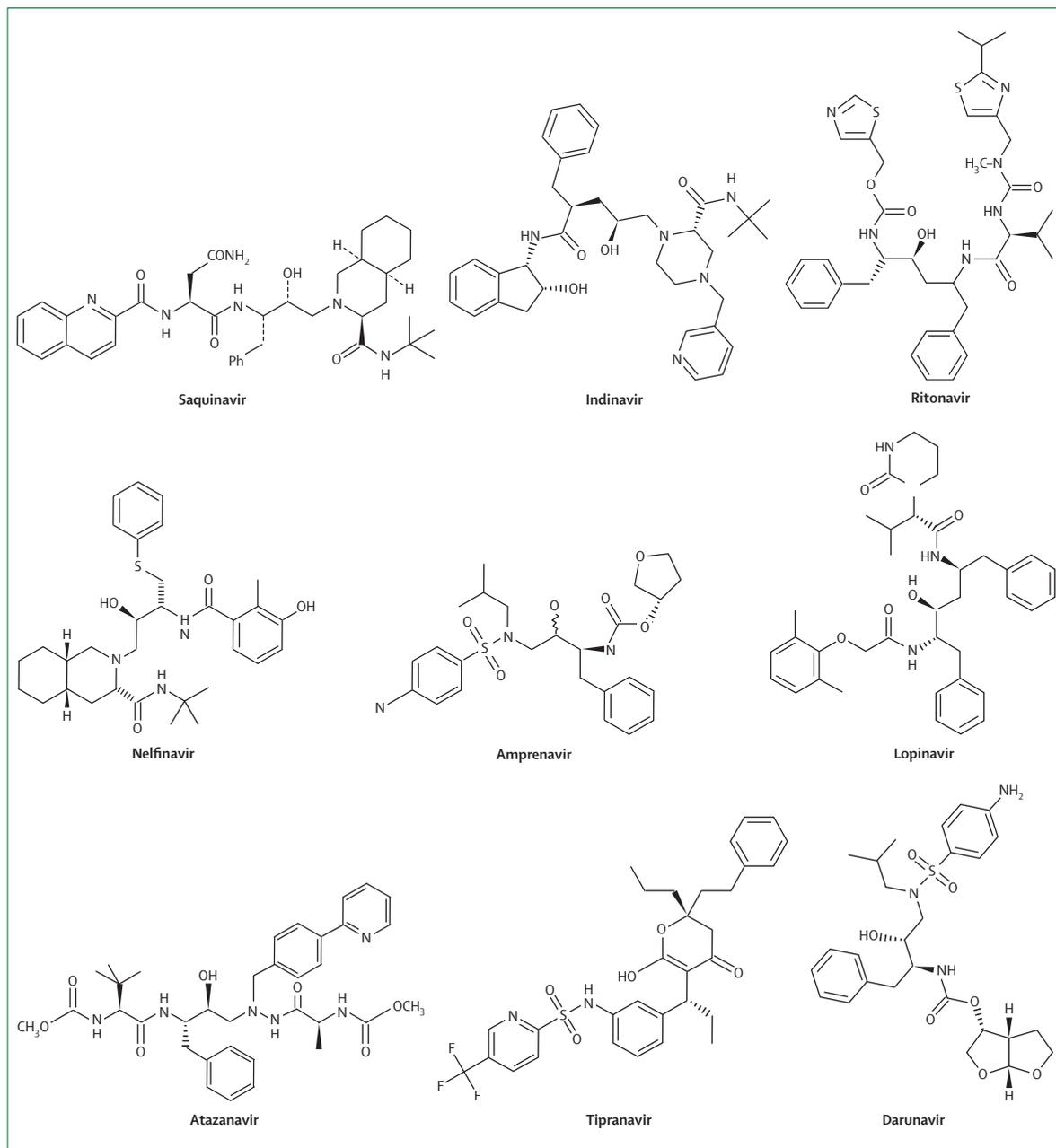


Figure 4: Chemical structures of FDA-approved protease inhibitors

Chemical structures of the HIV protease inhibitors approved by the US Food and Drug Administration are shown. Fosamprenavir (a prodrug of amprenavir) is not shown.

improving antigen presentation and activating T-cell responses, ritonavir inhibited non-immune 20S proteasome activity.⁴⁰ However, apart from ritonavir, only saquinavir inhibited chymotrypsin-like 20S proteasome activity, although much less efficiently, whereas no inhibition at all was observed with indinavir and nelfinavir.⁴⁰ These results clearly show that, although classified together therapeutically, HIV protease inhibitors are quite distinct compounds (table 1).

Saquinavir and Indinavir

Sgadari and colleagues^{37,41,42} first described the use of non-immune-mediated activity of HIV protease inhibitors against cancer; they showed that saquinavir and indinavir promoted regression of human Kaposi's sarcoma in nude mice. The HIV protease inhibitors did not inhibit proliferation of Kaposi's sarcoma or normal endothelial or smooth-muscle cells, rather they inhibited angiogenesis by blocking Kaposi's sarcoma cell invasion via inhibition of proteolytic activation of latent 72 kDa

type IV collagenase (also known as matrix metalloprotease 2 [MMP2]) to its active form. MMP2 is highly expressed in Kaposi's sarcoma and endothelial cells, and is necessary to degrade the basement membrane allowing endothelial-cell migration. Recombinant activated MMP2 had no direct inhibitory effects, which is consistent with the findings that MMP2 cleaves after a glycine, whereas the HIV protease is an aspartyl protease, and MMP2 has no sequence homology with the catalytic site of HIV protease. These effects were observed at low concentrations of both drugs (0.1–1.0 $\mu\text{mol/L}$). By contrast, at significantly higher drug concentrations (50–100 $\mu\text{mol/L}$), saquinavir inhibited 20S and 26S proteasome activity, increased apoptosis, and radiosensitised non-HIV-associated cancers, including prostate cancer, lymphoblastoid leukaemia, and glioblastoma.⁴³ These studies show clearly concentration-dependent cellular effects of HIV protease inhibitors.

Gupta and colleagues⁴⁴ reported that saquinavir (25 $\mu\text{mol/L}$)—in addition to amprenavir and nelfinavir but not indinavir or ritonavir—inhibited serine 473 Akt phosphorylation in H-ras mutated bladder cancer, epidermal growth factor receptor mutated head and neck cancer, and K-ras mutated pancreatic cancer and lung cancer cell lines. This resulted in radiosensitisation of these tumours in vitro and in vivo in a nude-mouse model. Importantly, overexpression of constitutively active phosphatidylinositol 3-kinase (PI3K), which lies upstream of Akt, in cells without activated Akt resulted in radiation resistance that could be overcome with the inhibitors of HIV protease. These results show that HIV protease inhibitors, such as saquinavir, might target a serine kinase between PI3K and Akt, possibly such as integrin linked kinase (ILK-1) or ataxia telangiectasia mutated.

Saquinavir might eventually have a role in treatment of imatinib-resistant chronic myelogenous leukaemia. Interestingly, an imatinib-resistant chronic myelogenous leukaemia cell line was more sensitive to saquinavir (inhibitory concentration₅₀ [IC₅₀] 6.0 $\mu\text{mol/L}$) than imatinib-sensitive cell line (IC₅₀ 43.9 $\mu\text{mol/L}$).⁴⁵ Furthermore, the addition of saquinavir 5 $\mu\text{mol/L}$ to imatinib reduced the IC₅₀ of imatinib in two imatinib-resistant chronic myelogenous leukaemia cell lines by over 18 and 37 times. This study shows the unique synergistic activity of saquinavir and an inhibitor of tyrosine kinase.

Indinavir was investigated in a murine model of hepatocellular carcinoma.⁴⁶ Similar to the effects in Kaposi's sarcoma, indinavir inhibited angiogenesis, which correlated with inhibition of MMP2 proteolytic activation. By contrast with Kaposi's sarcoma, induction of apoptosis was observed, whereas proliferation of the hepatocellular carcinoma cell lines was similarly unaffected. These same investigators subsequently did a phase I study of indinavir in dogs not with hepatocellular carcinoma, but rather

	Low concentration (<10 $\mu\text{mol/L}$)	High concentration (>10 $\mu\text{mol/L}$)
Saquinavir	Inhibition of MMP2 proteolysis Synergism with tyrosine kinase inhibitors	Inhibition of proteasome Inhibition of p-Akt
Indinavir	Inhibition of MMP2 proteolysis	Inhibition of proteasome
Ritonavir	Inhibition of cytokine production Inhibition of NF κ B activation Downregulate endoplasmic reticulum expression Inhibition of cdk 2, 4, 6, and cyclin D1 Inhibition of phospho-Akt Inhibition of Hsp90 Radiosensitisation Upregulation of Bax and inhibition of Bcl-2 Inhibition of CYP3A4 and synergism with docetaxel Inhibition of CYP24 and synergism with 1,25(OH) ₂ D ₃ Inhibition of MRP-1	Inhibition of proteasome
Nelfinavir	Inhibition of p-Akt Inhibition of STAT3 signalling Downregulation of androgen receptor Synergism with docetaxel Radiosensitization Decrease HIF-1 α and VEGF* Inhibition of CDK2 and Rb dephosphorylation Inhibition of SREBP-1 processing Upregulation of P21, Fas and Bax	Upregulation of P53 Upregulation of P21 and P27 Downregulation of Bcl-2 Downregulation of MMP2 Synergism with temozolomide Induce endoplasmic-reticulum stress-unfolded-protein response pathway Induce autophagy
Amprenavir	Inhibition of phospho-Akt	

VEGF=vascular endothelial growth factor.

Table 1: HIV-protease inhibitors and their proposed mechanisms of action

with spontaneous splenic haemangiosarcoma, an aggressive soft-tissue sarcoma that originates from malignant endothelial cells.⁴⁷ All three dogs in the first cohort died due to massive haemorrhage within 3 weeks of starting the study, and further dose-escalation was abandoned. Despite this caveat, Sgadari and colleagues^{41,42} developed a multicentre, phase II, clinical trial of indinavir for non-HIV-associated Kaposi's sarcoma in Italy, which has completed accrual and is undergoing analysis (ClinicalTrials.gov NCT00362310).^{41,42}

Ritonavir

Like saquinavir and indinavir, the antitumour activity of ritonavir was first shown in vitro for Kaposi's sarcoma and in vivo for a xenograft mouse model.⁴⁸ However, ritonavir inhibited proliferation of primary endothelial cells and induced apoptosis in Kaposi's sarcoma cell lines by several mechanisms, including inhibition of production of cytokines that contribute to neo-vascularisation (TNF α , interleukin 6, and vascular endothelial growth factor) and inhibition of transcriptional activation of NF κ B at clinically relevant concentrations (3–15 $\mu\text{mol/L}$). Despite previous report of 20S proteasome inhibition with ritonavir, there was no inhibition of chymotrypsin-like proteasome activity at the concentrations studied.^{40,48}

Similar to saquinavir, the pleiotropic effects of ritonavir seem to be concentration-dependent. At lower concentrations (2.5–40.0 $\mu\text{mol/L}$), ritonavir induced apoptosis and inhibited activation of NF κ B in primary adult T-cell leukaemia (ATL) cells.⁴⁹ Inhibition of NF κ B target gene

expression was also shown, including: Bcl-xL, baculoviral IAP repeat-containing protein 5 (survivin), c-Myc, and cyclin D2. Breast carcinoma is similarly sensitive to ritonavir.⁵⁰ The IC₅₀ of oestrogen-receptor-positive breast-cancer cell lines was 12–24 µmol/L, whereas the IC₅₀ of oestrogen-receptor-negative was higher (45 µmol/L). The increased sensitivity of oestrogen-receptor-positive breast carcinoma cells could be partly explained by ritonavir-mediated downregulation of oestrogen-receptor expression. In cell lines both negative and positive for oestrogen receptors, ritonavir induced G₁ arrest, reduced expression of cyclin-dependent kinases (cdk) 2, 4, and 6 and cyclin D1, induced apoptosis independent of G₁ arrest, inhibited Akt-phosphorylation, and inhibited the chaperone function of heat shock protein 90 (Hsp90).⁵⁰ At high concentrations of ritonavir (50–100 µmol/L), proliferation in glioma cells was inhibited by blocking G₁ and inducing apoptosis.⁵¹ At these higher concentrations, ritonavir inhibited 20S chymotrypsin proteasome activity with an IC₅₀ of 50 µmol/L. Finally, ritonavir inhibited growth of HEP2 head and neck carcinoma in nude mice at high concentrations (20–2000 µmol/L), and inhibition was synergistic when combined with radiation.⁵² Reduced proliferation and angiogenesis and increased apoptosis were associated with increased expression of Bax and decreased expression of Bcl-2. These studies suggest that the antitumour activity of ritonavir is broad but that the sensitivity to its effects is variable.

A promising avenue of research with ritonavir is exploiting its potential for synergism with other antitumour drugs. Ritonavir increased the anti-proliferative and proapoptotic effects of docetaxel in androgen-independent prostate cancer cell lines *in vitro* and *in vivo*.⁵³ Docetaxel induced the expression of cytochrome P450 3A4 (CYP3A4), a liver enzyme involved in metabolism of drugs, including docetaxel and HIV protease inhibitors, and 10 µmol/L ritonavir completely blocked this induction. Ritonavir also potentiates the effect of 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] to induce growth arrest and differentiation of human myeloid leukaemia cells via down-regulation of cytochrome P450 24A (CYP24A), which converts [1,25(OH)₂D₃] to its inactive form 1,24,25,(OH)₃.⁵⁴ Additionally, ritonavir inhibited the functional activity of the multidrug resistance-associated protein 1 (MRP1), a cellular efflux pump on the plasma membrane important for transport of drug out of the cell in an ATP-dependent fashion.⁵⁵ Some cytotoxic chemotherapeutic drugs, as well as HIV protease inhibitors, are substrates of MRP1. Inhibition of CYP3A4 and MRP1 are currently being exploited with ritonavir boosting, in which ritonavir improves the pharmacokinetics of other HIV protease inhibitors.⁵⁶ Cytotoxic chemotherapy might be combined with ritonavir clinically to increase the area under the curve of chemotherapy exposure.

Nelfinavir

Nelfinavir may have the most potent antitumour activity of the HIV protease inhibitors.^{57–59} Screening six FDA-approved inhibitors in cell-proliferation assays with two non-small-cell lung cancer cell lines, Gills and colleagues⁵⁹ found that nelfinavir was the most potent. This finding was confirmed in the NCI60 cell-line panel that contains 60 cell lines derived from nine different tumour types.⁵⁹ The average IC₅₀ concentration was 5.2 µmol/L, and cytotoxicity was observed in 14 of 60 cell lines at doses 10 µmol/L or less.

Like other HIV protease inhibitors, nelfinavir has numerous cellular effects that might account for its broad antitumour activity. Yang and co-workers⁶⁰ showed that nelfinavir inhibited growth and induced apoptosis in cell lines of androgen-dependent and androgen-independent prostate cancer, via disrupted signal transducer and activator of transcription 3 (STAT3) signalling at 10–50 µmol/L. Nelfinavir also blocked androgen receptor signalling and downregulation of the androgen receptor in androgen-dependent prostate cancer cells. Furthermore, nelfinavir inhibited growth of androgen-independent prostate tumours without significant toxic effects in a model with nude mice. The same investigators also showed similar inhibitory and proapoptotic effects in non-small-cell lung cancer via upregulation of P53 and the cyclin-dependent kinase inhibitors P21 and P27 and downregulation of Bcl-2 and MMP2.⁵⁸ These effects might have been mediated through inhibition of Akt phosphorylation, as forced expression of constitutively active Akt partly reversed the nelfinavir-mediated growth inhibition. Finally, nelfinavir increased docetaxel-mediated inhibition of proliferation.

Nelfinavir is a potent radiosensitiser. The PI3K–Akt pathway upregulates vascular endothelial growth factor and hypoxia-inducible factor 1α, so nelfinavir-mediated inhibition of Akt phosphorylation decreased hypoxic induction and inhibited angiogenesis in head and neck carcinoma and non-small-cell lung carcinoma.⁶¹ As a result, the combination of nelfinavir and radiation, compared with radiation alone, increased time to regrowth of a non-small-cell lung cancer xenograft mouse model. Nelfinavir also decreased phosphorylation of Akt, increased radiosensitisation, and increased chemoradiation sensitivity with temozolomide in glioblastoma cells deficient in phosphatase and tensin homologue.⁶² Nelfinavir did not decrease phosphorylation of Akt in immortalised human astrocytes or radiosensitise them, thus showing the drugs selectivity for malignant cells.

Jiang and colleagues⁶³ showed that nelfinavir might also inhibit growth of melanoma cells by inducing G₁ cell-cycle arrest. However, in this instance, the mechanism was through inhibition of cell division protein kinase 2 (CDK2) and dephosphorylation of retinoblastoma protein with IC₅₀ concentration between 5–12 µM.⁶³ Nelfinavir inhibits CDK2 through activation of proteasome-

dependent degradation of Cdc25A phosphatase. Ritonavir, by contrast, has a primary mechanism of proteasome inhibition at high concentrations. Also contrary to other reports, in Jiang and colleagues study, nelfinavir did not inhibit Akt signalling, but rather activated it. Because the PI3K–Akt pathway promotes survival, the investigators speculated that activation of Akt signalling reflected the cellular response to the stressed condition induced by nelfinavir.

A new antitumour mechanism of action for nelfinavir has recently been described. Nelfinavir induces malignant cell death by inducing overwhelming protein misfolding in the endoplasmic reticulum (endoplasmic-reticulum stress).^{59,64} Surface and secreted proteins are synthesised in the endoplasmic reticulum where they must fold and assemble properly before being transported. Cells respond to endoplasmic-reticulum stress by activating an unfolded-protein response, which reduces synthesis of new proteins to relieve the load on the stressed endoplasmic reticulum and upregulates genes that promote the endoplasmic reticulum's capacity to cope with unfolded and misfolded proteins in the long term. When the capacity of the unfolded-protein response is exceeded, the cell simply undergoes caspase-dependent apoptosis.⁶⁵ Accordingly, Gills and colleagues⁵⁹ and Pyrko and co-workers⁶⁴ reported that nelfinavir activates the endoplasmic reticulum stress–unfolded-protein response pathway in non-small-cell lung carcinoma and glioma cells. Additionally, Gills and colleagues⁵⁹ clarified the controversial role of Akt inhibition; it seems that the kinetics of inhibition were cell-line specific. Finally, the researchers investigated the role of autophagy in nelfinavir-mediated cellular effects. Autophagy is a process in which intracellular membrane structures sequester proteins and organelles to degrade and turn over these materials under conditions of stress, starvation, or inhibition of the PI3K–Akt–mammalian target of rapamycin pathway.^{64,66} The researchers showed that induction of autophagy counteracts the cytotoxicity of nelfinavir, because inhibiting autophagy with a small-molecule inhibitor of autophagy (3-methyladenine) increased nelfinavir-induced death.⁵⁹

Our interest in HIV protease inhibitors developed as a result of the observation of lipodystrophy syndrome in patients with HIV treated with protease inhibitors in the HAART era.⁶⁷ In this syndrome, peripheral lipoatrophy and central fat accumulation develop in association with insulin resistance and hyperlipidaemia. Although the lipodystrophy syndrome is associated not only with HIV protease inhibitors, but also, more commonly, with nucleoside reverse transcriptase inhibitors, particularly stavudine, a role of HIV protease inhibitors has not been entirely eliminated.⁶⁸ Pathogenesis of lipodystrophy syndrome has been linked to sterol regulatory element binding protein-1 (SREBP-1), a key adipocyte differentiation and lipogenic transcription factor, which is produced as an inactive precursor tethered to the

endoplasmic reticulum by a hydrophobic tail.⁶⁹ During periods of cholesterol depletion, proteolytic removal of the tail in two sequential cleavage steps by site-1 and site-2 proteases releases active SREBP-1 to travel to the nucleus, and activates genes involved in long-chain fatty-acid synthesis and adipocyte differentiation in a process known as regulated intramembrane proteolysis.⁷⁰ In mice fed 50 μ L ritonavir (2 mg/day) for 10 days, the lipodystrophy phenotype was reproduced by accumulation of activated SREBP in the nucleus, and in transgenic mice engineered to overexpress the protein, the lipodystrophy phenotype was recapitulated.^{71,72} Furthermore, analysis of peripheral adipose tissue from patients with HIV receiving HAART showed increased SREBP-1 content despite a 93% reduction in SREBP-1 mRNA.⁷³ These intriguing studies stimulated our hypothesis that inhibitors of HIV protease might be particularly effective antitumour drugs in liposarcoma, a tumour of adipocyte origin that expresses SREBP-1.

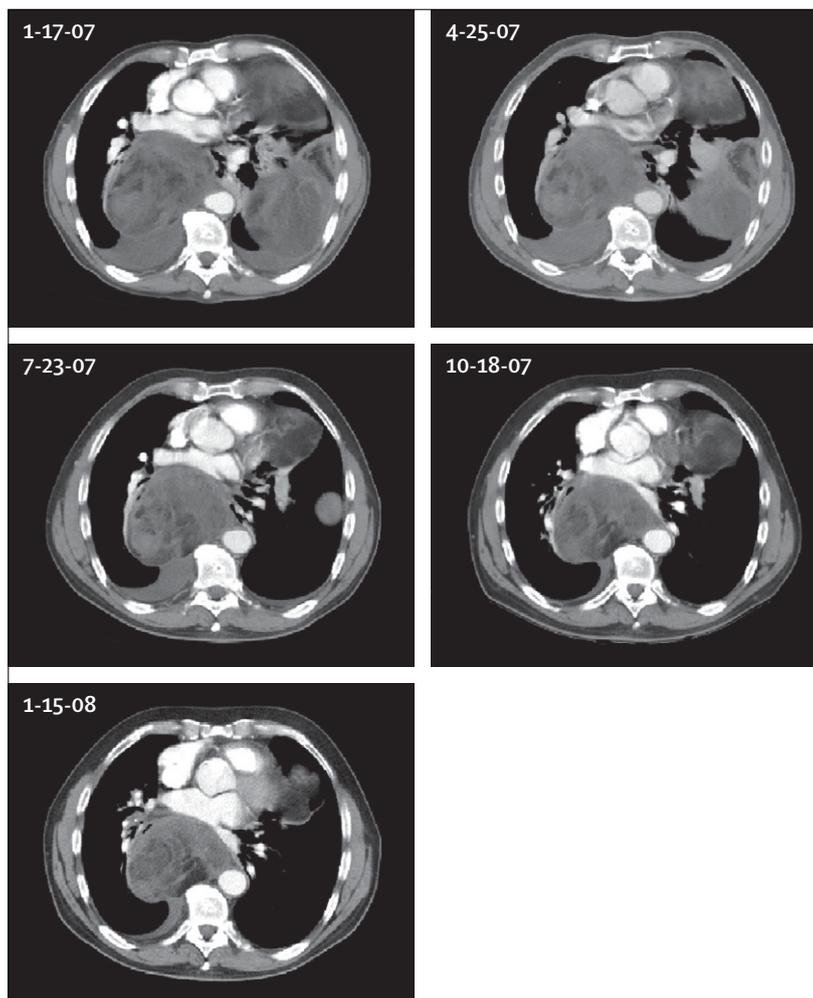


Figure 5: Serial chest CT scans of a patient in a clinical trial of nelfinavir for liposarcoma. 64-year-old male with a dedifferentiated liposarcoma unresponsive to combination chemotherapy treated with nelfinavir 1500 mg orally twice daily beginning Feb 8, 2007. ClinicalTrials.gov ID NCT00233948.

We showed that nelfinavir was the most potent protease inhibitor (nelfinavir, ritonavir, saquinavir, indinavir, and amprenavir); it inhibited colony formation in clonogenic assays in liposarcoma cells while sparing fibrosarcoma and embryonic kidney cells.⁵⁷ Nelfinavir led to increased concentrations of precursor and mature SREBP-1 selectively in liposarcoma cells. Wang and co-workers⁷⁴ showed that antiproliferative P21 and proapoptotic Fas and Bax are direct targets of SREBP-1, therefore we investigated their expression in liposarcoma cells. Nelfinavir induced expression of these proteins, which resulted in induction of G₁ arrest and apoptosis. Finally, forced expression of SREBP-1, in the absence of nelfinavir produced the molecular and biological phenotype of nelfinavir treatment. Recent studies showed that the half-life of precursor SREBP-1 is longer in cells treated with nelfinavir treated than in untreated cells (unpublished). Nelfinavir might inhibit proper regulated intramembrane proteolysis of SREBP-1 in liposarcomas, which could elicit the endoplasmic reticulum stress–unfolded protein response pathway. These studies provided the impetus for a phase I/II clinical trial of nelfinavir for liposarcomas (NCT00233948).⁷⁵ Of 11 evaluable patients, four had stable disease after at least 3 months of therapy, and one developed a confirmed partial remission. A 64 year-old man had an unresectable, intermediate-grade, de-differentiated liposarcoma of the left hemithorax refractory to two lines of combination chemotherapy. After treatment at with 1500 mg nelfinavir orally twice daily for 12 months, he had partial remission (figure 5).⁷⁵ The FDA-approved dose of nelfinavir for treatment of HIV infection is 1250 mg orally twice daily; however, this study shows the potential of nelfinavir in cancer treatment.

In addition to our ongoing trial, there are two other ongoing US clinical trials of nelfinavir for cancer as a single-agent or in combination with chemotherapy and radiation (table 2). A phase I trial of nelfinavir in combination with a fixed dose of cisplatin and escalating doses of gemcitabine in combination with radiation for locally advanced pancreatic cancer was recently reported.⁷⁶ Partial responses were observed in five of ten patients who completed chemoradiotherapy, and secondary complete resection was possible in six of the ten patients where a complete remission was observed in one patient. As a basis of comparison for this small series, the secondary resection rate after chemoradiotherapy in patients with the same eligibility criteria as in the current study at the same institution was 20%. This study adds to the growing body of evidence for the potential of nelfinavir in cancer treatment.

Lopinavir, atazanavir, tipranavir, and darunavir

Lopinavir has insufficient bioavailability when given alone; however, like other HIV protease inhibitors, its blood concentrations are greatly increased by low-dose ritonavir.⁵⁶ Lopinavir inhibited the growth of cervical carcinoma cell lines infected with human papilloma virus 16 by inhibiting viral E6-activation of proteasomal degradation of nuclear P53 and subsequent induction of apoptosis.⁷⁷ Atazanavir is less likely to cause lipodystrophy and hyperlipidaemia, but, like lopinavir, requires boosting with ritonavir, which reduces its metabolic advantage. Atazanavir, like nelfinavir, activates the endoplasmic reticulum stress–unfolded-protein response pathway in glioma cells.⁶⁴

Second generation HIV-protease inhibitors include tipranavir and darunavir. Tipranavir is the first non-peptidomimetic approved by the FDA for treatment of

Cancer type and stage	Trial phase	Institution	Treatment and schedule	
NCT00233948	Recurrent liposarcoma	I/II	City of Hope, Duarte, CA, USA	Nelfinavir orally twice daily every 28 days
NCT00436735	Solid tumours	I	National Cancer Institute, Bethesda, MD, USA	Nelfinavir orally twice daily every 21 days
NCT00589056	Stage III NSCLC	I/II	University of Pennsylvania, Philadelphia, PA, USA	Nelfinavir orally twice daily beginning 1–2 weeks before initiation of chemoradiotherapy and continuing until completion of radiotherapy 5 days per week for 7–8 weeks; with intravenous cisplatin intravenously days 1, 8, 29, and 36 and etoposide intravenously days 1–5 and 29–33
NCT00705393	Locally advanced pancreatic cancer	I	University of Nebraska, Omaha, NE, USA	Nelfinavir orally twice daily concurrent with stereotactic radiotherapy after neoadjuvant gemcitabine, fluorouracil, and calcium folinate chemotherapy, followed by surgery and adjuvant chemotherapy
NCT00704600	Rectal cancer	I/II	Maastricht Clinic, Maastricht, Netherlands	Nelfinavir orally twice daily beginning 7 days before chemoradiotherapy with capecitabine 825 mg/m ² twice daily
NCT00694837	Glioblastoma	I/II	Maastricht University, Maastricht, Netherlands	Nelfinavir orally twice daily beginning 7 days before chemoradiotherapy with temozolomide
NCT00591682	Solid tumors	I	University of Texas MD Anderson Cancer Center, Houston, TX, USA	MSX-122 (CXCR4 antagonist) orally once daily every 28 days
NCT00512252	Acute myelogenous leukemia	I/II	Washington University St Louis, MO, USA	AMD3100 (CXCR4 antagonist) with mitoxantrone, etoposide, and cytarabine

NSCLC=non-small-cell lung cancer. Clinical trials investigating the toxicities, best dose, and efficacy of different schedules of nelfinavir (with and without chemoradiation) and CXCR4 antagonists for various cancers are shown.

Table 2: Clinical trials investigating anti-HIV drugs for cancer

HIV infections, including those resistant to peptidomimetic HIV protease inhibitors.⁷⁸ Darunavir is the most recent HIV protease inhibitor to receive FDA approval. Darunavir was designed to form robust interactions with the protease enzyme from many strains of HIV, including strains from patients with multiple resistance mutations to HIV protease inhibitors.⁷⁹ Currently, the anticancer potential of tipranavir and darunavir remain unreported.

Chemokine-receptor antagonists

Chemokines are secreted factors first described as regulators of leucocyte trafficking during inflammation.⁸⁰ Chemokine CXCL12 binds its cognate cellular receptor CXCR4. Chemokine receptors CXCR4 and CCR7 are present in several types of cancer and involved in progression and metastasis.⁸¹ CXCR4 is an important co-receptor for entry of T-tropic (X4) HIV in addition to CD4 for membrane fusion and entry into the cell, and CXCR4 antagonists have been developed for HIV therapy.⁸² HIV drugs that target CXCR4 might, therefore, be useful in cancer therapy. AMD 3100, a small-molecule antagonist of CXCR4 in preclinical assessment for HIV treatment, inhibits intracranial growth of primary brain tumours in glioblastoma and medulloblastoma murine xenografts.⁸³ Analogues of another CXCR4 antagonist, T140, inhibit migration of breast cancer and endothelial cells in vitro, and reduce pulmonary metastases in immunodeficient mice inoculated with breast cancer cells in vivo.⁸⁴ A separate analogue of T140, 4F-bTE, had similar in-vitro inhibition of migration and invasion in bladder cancer.⁸⁵ These studies show the potential application of chemokine receptor antagonists for cancer treatment (table 2).

Conclusion

Development of new drugs for cancer therapy is a lengthy and costly endeavour. In addition to the obvious financial benefits, the repositioning of an approved drug for cancer therapeutics has other advantages, such as implicit knowledge of its toxicity profile, drug metabolism, pharmacokinetics, and drug interactions. Although nucleoside analogues, such as zidovudine, didanosine, and ganciclovir, have very limited roles in non-HIV-related cancer treatment, non-nucleoside reverse transcriptase inhibitors, CXCR4 antagonists, and especially HIV-protease inhibitors, such as nelfinavir, hold promise as antineoplastic drugs. The HIV protease inhibitors possess pleiotropic cellular effects that lead to antitumour activity, including inhibition of MMP2 activation, NF κ B activation, proteasome activity, Akt phosphorylation, production of proangiogenic factors, STAT3 activation, and SREBP-1 processing; they also induce endoplasmic-reticulum stress. Importantly, none are mutually exclusive of another, and the effects are protease-inhibitor-dependent. As the mechanisms of their antitumour activity continue to be investigated, other mechanisms

Search strategy and selection criteria

Data for this review were identified by searches of PubMed, Medline, and references from relevant articles with the search terms "anti-HIV agents" and "cancer". Additional references were selected from relevant articles. Abstracts and reports from meetings were included only when they related directly to previously published work. Only papers published in English between January, 1992, and June, 2008, were included.

will no doubt be discovered. And as early clinical trials of HIV protease inhibitors for cancer mature, the full oncological potential of these drugs might be realised. Clearly, a debt is owed to HIV research for successes in bringing these drugs into the clinical arena.

Conflicts of interest

The authors' institution, City of Hope, Duarte, CA, USA, have filed a patent application for nelfinavir in treatment of liposarcoma. The authors declared no other conflicts of interest.

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