REVIEW

REVISED Nelfinavir and other protease inhibitors in cancer: mechanisms involved in anticancer activity [version 2; peer review: 2 approved]

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Abstract

Objective: To review the mechanisms of anti-cancer activity of nelfinavir and other protease inhibitors (PIs) based on evidences reported in the published literature.

Methods: We extensively reviewed the literature concerning nelfinavir (NFV) as an off target anti-cancer drug and other PIs. A classification of PIs based on anti-cancer mode of action was proposed. Controversies regarding nelfinavir mode of action were also addressed.

Conclusions: The two main mechanisms involved in anti-cancer activity are endoplasmic reticulum stress-unfolded protein response pathway and Akt inhibition. However there are many other effects, partially dependent and independent of those mentioned, that may be useful in cancer treatment, including MMP-9 and MMP-2 inhibition, down-regulation of CDK-2, VEGF, bFGF, NF- κ B, STAT-3, HIF-1 α , IGF, EGFR, survivin, BCRP, androgen receptor, proteasome, fatty acid synthase (FAS), decrease in cellular ATP concentration and upregulation of TRAIL receptor DR5, Bax, increased radiosensitivity, and autophagy. The end result of all these effects is slower growth, decreased angiogenesis, decreased invasion and increased apoptosis, which means reduced proliferation and increased cancer cells death. PIs may be classified according to their anticancer activity at clinically achievable doses, in AKT inhibitors, ER stressors and Akt inhibitors/ER stressors.

Beyond the phase I trials that have been recently completed, adequately powered and well-designed clinical trials are needed in the various cancer type settings, and specific trials where NFV is tested in association with other known anti-cancer pharmaceuticals should be sought, in order to find an appropriate place for NFV in cancer treatment.

The analysis of controversies on the molecular mechanisms of NFV hints to the possibility that NFV works in a different way in tumor cells and in hepatocytes and adipocytes.

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Keywords

Nelfinavir, protease inhibitor, cancer, endoplasmic reticulum stress, unfolded protein response, Akt

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REVISED Amendments from Version 1

In this version, a table was included listing past and ongoing clinical trials using nelfinavir in cancer treatment.

See referee reports

Abbreviations

NFV: Nelfinavir

PI: HIV Protease Inhibitors

ERS: Endoplasmic reticulum stress

UPR: Unfolded protein response

BCRP: Breast cancer resistance protein

FAS: Fatty Acid Synthase

Introduction

In March 1997, the United States Food and Drug Administration (FDA) approved Nelfinavir (NFV, brand name Viracept) for HIV treatment in humans¹. NFV is a safe, orally available, and potent drug against HIV-1 and HIV-2². This protease inhibitor (PI) was developed by the private pharmaceutical sector and was a big success in the treatment of AIDS in association with other anti-retroviral drugs³. The introduction of PIs combined with HIV reverse transcriptase inhibitors started the era of HAART (highly active anti-retroviral treatment) and is nowadays the standard of care in HIV-AIDS⁴.

Pis inhibit HIV-1 and HIV-2 proteases (which are aspartate proteases), impeding virus replication and release of infecting viral particles from diseased cells. The mechanism of action of protease inhibitors involves competitive binding to the enzyme⁵.

NFV is being progressively displaced from HIV therapeutics by second generation HIV PIs, but has shown interesting off target actions in cancer.

The possible use of anti-HIV drugs against cancer is not new: in the 1990s AZT (zidovudine or azidothymidine) was proposed as anti-neoplastic drug, but clinical trials did not confirm the preliminary good results obtained *in vitro*⁶.

That HIV PIs target other molecules besides the HIV protease is quite evident if we examine adverse effects like insulin resistance and lipodystrophy. These and other evidences such as inhibition of tumor cell production of cytokines, anti-angiogenesis, induction of apoptosis and others, suggest off targets effects for PIs, and hints to the concept of a new class of drugs against cancer with multiple anti-cancer effects⁶.

NFV, the most important anti-cancer drug of the PI family, if repurposed for cancer treatment, would have an important advantage: it has been used for more than 15 years in HIV treatment and its safety, pharmacokinetics, and adverse events are well known. Serious adverse events are not common with the exception of diarrhea when used at high doses.

Research on NFV as a potentially useful drug for cancer treatment⁶ started in 2009.

In this article, we thoroughly review the literature published in this matter and analyze mainly the anti-cancer mechanisms of action of NFV.

Certain controversies regarding NFV activity in lipid metabolism will be considered in depth.

Evidences of nelfinavir anti-cancer activity

A partial response of Kaposi's sarcoma patients to PIs was published in 1998⁷ and good results with regression (six complete responses out of 10 patients)⁸. In 1999 Niehues *et al.*⁹ published complete regression of Kaposi's sarcoma in a child treated with highly active anti-retroviral therapy (HAART). Sgadari *et al.* (2003) described also the inhibition of Kaposi's sarcoma with protease inhibitors and they also mention that these drugs can antagonize vital properties of tumor cells like growth, invasion, tissue remodelling, angiogenesis and survival. They consider these effects to be a consequence of inhibition of invasion, matrix metalloprotease, proteasome and NF- κ B signaling¹⁰. The possible mechanisms of PIs off target activity on tumor cells were described by pioneering work of Schmidtke *et al.* in 1999¹¹: they observed that ritonavir was a modulator of proteasomal activity, allowed normal proliferation when used at low concentrations, but affected protein degradation when present at higher concentrations, and cell cycle was arrested.

Ikezie *et al.*¹² described that protease inhibitors increased cellular growth inhibition of all transretinoic acid (ATRA) on cell cultures of myelocytic leukaemia lines. Protease inhibitors also increased differentiation of acute myeloid leukemia cell lines.

In 2004, Ikezie¹³ described the mechanisms involved in anti-cancer activity of protease inhibitors in myeloma cells.

The mechanisms involved in PIs anti-cancer activity are summarized in chronological order on [Table 1](#).

Nelfinavir and the ERS-UPR pathway

NFV inhibits the proteases S1P and S2P that are involved in SREBP-1 maturation and other proteases necessary for protein maturation and folding (yet not fully identified) in the endoplasmic reticulum⁴⁹.

Activation of the unfolded protein response (UPR) starts in the ER when abnormal accumulation of protein is detected⁵⁷. This was investigated thoroughly in yeasts where detection of abnormal protein occurs through Ire1p/Ern1p-mediated signaling from the ER (in mammals there are three sensor proteins IRE1 α , PERK and ATF6⁵⁸). UPR activation leads to the specific removal of 252 nucleotides intron from a precursor mRNA of the transcription factor HAC-1p, and the resulting mature mRNA HAC-1p is translated to produce active HAC-1p. This transcription factor translocates to the nucleus and promotes the transcription of chaperones like GRP78 that facilitates removal of abnormal proteins from the ER through retrotranslocation and final disposal by the ubiquitin-proteasome pathway⁵⁹.

HAC1 precursor mRNA is constitutively expressed but not translated until Ire1p/Ern1p sensor removes the necessary nucleotides.

Thus the UPR is an intracellular signaling pathway where the ER “informs” the nucleus on the need to increase the levels of molecular chaperones and folding enzymes in order to maintain the ER homeostasis. Therefore UPR keeps unfolded proteins in the ER until they are correctly folded before they can go to their final destination. NFV seems to produce cellular stress by

accumulation of misfolded or abnormal proteins in the ER, overwhelming the normal ER protein folding machinery⁶⁰. Chaperones bound to unfolded proteins in the ER initiate protein kinase cascades that inhibit translation, reverse translocation, activate ubiquitination enzymes, induce autophagia, and when stress is extreme, induce apoptosis.

Table 1. Mechanisms of action of Nelvinavir and other PIs in cancer.

Author	Study performed in	Results
Andre, 1998 ¹⁴	Mice infected with lymphocytic choriomeningitis virus receiving ritonavir.	Ritonavir inhibits chymotrypsin-like activity of the 20S proteasome. Nelfinavir does not inhibit chymotrypsin like activity.
Gaeddicke, 2002 ¹⁵	Thymoma cells growing in syngeneic mouse	Ritonavir produces growth inhibition of tumors, apoptosis and affects proteosomal proteolysis. Non-transformed cell lines were relatively resistant to this activity. Accumulation of p21 (due to inhibition of proteolytic degradation).
Ikezoe, 2004 ¹³	Human Multiple Myeloma cells	Growth arrest, apoptosis, blocked IL6 stimulated phosphorylation of STAT 3 and ERK 1/2. Decreased VEGF production.
Sgadari, 2002 ¹⁶	Kaposi sarcoma cell lesions in nude mice	Anti-angiogenesis. Decrease of VEGF, bFGF. Decrease MMP2 activation.
Pajonk, 2002 ¹⁷	PC-3 and DU-145 prostate cancer, U373 glioblastoma, and K562 and Jurkat leukemia cells	Saquinavir inhibited activation of NF-κB. Inhibited 20s and 26s proteasome activity. Sensitized the surviving cells to ionizing radiation. In a previous paper ¹⁸ , this same group showed that proteasome is a direct target of radiation. This explains the synergism between proteasome inhibitors and ionizing radiation.
Olson, 2002 ¹⁹	Cell culture of UMCC-1/VP cells which over-express MRP-1	Ritonavir inhibits the functional activity of the multidrug resistance related-protein 1 (MRP-1). This characteristic was not shared by other PIs.
Zhou, 2004 ²⁰	Insulinoma cells	Nelfinavir decreased insulin stimulated phosphorylation of IRS-2 and Akt-Thr(308) in a dose-dependent manner. For 10 micromol/L of nelfinavir, the decrease in Akt phosphorylation was 55%.
Gupta, 2004 ²¹	Human embryonic kidney cells	HIV PIs are breast cancer resistance protein inhibitors. This applies to ritonavir, saquinavir, and nelfinavir. Indinavir and amprenavir showed no inhibition on BCRP.
Piccinini, 2005 ²²	HL60 cells incubated with and without drug.	Saquinavir and nelfinavir inhibited proteasome activity at therapeutic dosages. Retroviral medication had no effect on proteasome.
Gupta, 2005 ²³	Tumor cell culture and xenografts.	Amprenavir, nelfinavir, and saquinavir inhibited Akt phosphorylation and exerted synergistic effects with radiotherapy.
Yang, 2005 ²⁴	Prostate Cancer Cells: LNCaP, DU145, PC3 and LNCaP xenografts in nude mice	NFV induces growth arrest and apoptosis of prostate cancer cells and blockade of androgen receptor, STAT3 and AKT. It also inhibits proliferation of LNCaP xenografts.
Yang, 2006 ²⁵	NSCLC cell culture and xenografts in nude mice.	NFV induces growth arrest, reduces Akt signalling, apoptosis and docetaxel sensitisation. It is responsible for up-regulation of p21, p27 and p53, down-regulation of Bcl-2 and MMP-2. NFV slowed proliferation and induced apoptosis in tumour xenografts mice without adverse systemic effects. Of the 3 PIs tested (saquinavir, ritonavir and NFV) NFV exerted the strongest inhibition on proliferation.
Chow, 2006 ²⁶	Liposarcoma and non liposarcoma cell lines	NFV induces apoptosis of liposarcoma cell through upregulation of SREBP-1. Authors consider that NFV is a new class of anti-liposarcoma agent.
Pore, 2006 ²⁷	Glioblastoma cells	NFV decreased VEGF expression and secretion under normoxia. NFV decreases VEGF through the PI3K/Akt pathway. NFV also decreased the hypoxic induction of VEGF and the hypoxic induction of HIF-1α. NFV's effect was a decreased angiogenesis
Pore, 2006 ²⁸	<i>In vivo</i> Matrigel plug assay	NFV decreases VEGF expression through the transcription factor Sp1, which regulates VEGF promoter. It down-regulates HIF-1 α by decreasing translation.
Hampson, 2006 ²⁹	HPV transformed cervix carcinoma cells	Protease inhibitors inhibit S 26 proteasome blocking p53 degradation.
Ben-Romano, 2006 ³⁰	Cell culture of 3T3-L1 adipocytes	NFV induces oxidative stress that may lead to apoptosis (in adipocytes).
Gupta, 2007 ³¹	Meningioma cells	Combination therapy with imatinib and NFV potentiated anti-proliferative activity of imatinib due to decrease in survivin and increase of Bax.

Author	Study performed in	Results
Jiang, 2007 ³²	Melanoma cells	NFV produces cell cycle arrest and apoptosis through inhibition of CDK2.
Jiang and Pore, 2007 ³³	Glioblastoma cells	NFV decreased Akt expression and enhanced radiosensitization in PTEN deficient glioblastoma cells.
Gills, 2007 ³⁴	NSCLC xenografts and breast cancer resistant cell lines	NFV induced caspase dependent apoptosis and also caspase independent apoptosis via ER stress and autophagy.
Cuneo, 2007 ³⁵	HUVEC and tumor vascular endothelium	NFV enhance the effects of irradiation on endothelial cells.
Pyrko, 2007 ³⁶	Glioblastoma cell lines	Endoplasmic reticulum stress (ERS) response, as shown by increased expression of ERS markers, GRP78 and CHOP, and activation of ERS-associated caspase-4. Proteasome inhibition.
De Barros, 2007 ³⁷	Human subcutaneous abdominal white adipose tissue	PIs inhibited proteasome and differentiation of human preadipocytes in culture, reducing expression and production of matrix metalloproteinase 9 (MMP-9).
Gills, 2008 ³⁸	60 different cancer lines	NFV causes apoptosis and non-apoptotic death, ERS and autophagy. Blocks growth factor receptor activation and decreases growth factor-induced and endogenous Akt signaling. <i>In vivo</i> , NFV inhibits tumor growth.
Plastaras, 2008 ³⁹	Leucocytes of HIV patients receiving PI (peripheral blood biomarker assay)	Decreased Akt activation at clinically achievable doses. Increases sensibility of cancer cells to radiotherapy. PIs do not increase toxicity in patients receiving radiotherapy.
Brüning, 2008 ⁴⁰	Ovarian cancer cells	NFV upregulates TRAIL receptor DR5 which is an apoptosis inducing receptor.
Giri, 2009 ⁴¹	MDCKII wild-type and Bcrp1-transfected cell lines	NFV may act as a breast cancer resistance protein (BCRP) inhibitor with certain substrates.
Brüning, 2009 ⁴²	Ovarian cancer cell lines. Ascites samples of cancer patients.	NFV induced cell death in carboplatin-sensitive resistant ovarian cancer cell lines. NFV induced formation of ER-derived vacuoles and induced up-regulation of the hsp70 heat shock family member GRP78. It induced the unfolded protein response, which causes cell cycle arrest and apoptosis. Down-regulation of cell cycle regulatory proteins, especially cyclin D3.
Dewan, 2009 ⁴³	Lymphoblastoid B cells <i>in vitro</i> and in mice model	Ritonavir induced cell cycle arrest and apoptosis by down-regulation of cyclin D2 and surviving and suppressed transcriptional activation of NF- κ B.
Wang, 2010 ⁴⁴	Tumor vascular network	NFV improved vascular network.
Xie, 2011 ⁴⁵	Chemical systems biology	Weak inhibition of multiple kinases is one of the causes of NFV anti-cancer activity, without severe side effects, but still having an impact on the system. Off targets of NFV are possibly: EGFR, IGF-1R, Akt2, Abl, FGFR, CDK2, ARK2, Fak1, PDK1, Ephrin receptors. The concept behind this research is that the whole is greater than the sum of the parts.
Tian, 2011 ⁴⁶	Glioblastoma cells	Authors describe a pathway NFV/ERS/CHOP/up regulation of trail receptor DR5. This pathway clearly interconnects NFV with apoptosis.
Brüning, 2011 ⁴⁷	Cervical cancer line	NFV and Bortezomib (a proteasomal inhibitor) show synergy as apoptosis inducers.
Zeng, 2011 ⁴⁸	Pituitary adenoma cells and xenografted tumors	Growth retardation <i>in vivo</i> . Inhibition of PI3K-Akt-mTOR axis. Increased sensitivity to radiation.
Guan, 2012 ⁴⁹	Cell culture	NFV inhibits proteolysis of SREBP-1 by inhibiting site-2 protease (S2P). Inhibition of autophagy with hydroxychloroquine enhanced apoptotic effect of nelfinavir. Accumulation of SREBP-1 produced ER stress. Decreases FAS.
Bono, 2012 ⁵⁰	Human myeloma plasma cells and xenografted SCID mice	Inhibition of S26 proteasome, impaired proliferation and increased apoptosis. Decreased phosphorylation of AKT, STAT3 and ERK 1/2. ER stress corroborated by PERK and CHOP.
Brüning, 2012 ⁵¹	HeLa cells and other cancer cells	NFV and other ER stressor upregulate inhibin Beta E which shows anti-proliferative effects.
Barillari, 2012 ⁵²	Cervical intraepithelial neoplasia cells	Saquinavir and ritonavir reduce MMP2 and 9, inhibiting cell invasion and growth.
Timeus, 2012 ⁵³	Neuroblastoma cells	Saquinavir showed anti-proliferative and anti-invasive activity which was increased by the association with imatinib. Activation of NF- κ B was decreased. Saquinavir also exerted a pro-apoptotic activity on NB lines, which was significantly increased by the association with imatinib.
Ismail, 2013 ⁵⁴	Hamster ovary cells	Inhibits proximal insulin receptor signalling which may explain insulin resistance.
Escalante, 2013 ⁵⁵	Myeloma cells in culture and mice xenografts.	NFV is a calpain blocker. This activity enhances bortezomib (a proteasomal inhibitor) cytotoxicity <i>in vivo</i> and <i>in vitro</i> . Drugs that block the HIV1-associated aspartyl protease also show cross reactivity with the cysteine protease calpain.
Bociaga-Jasik, 2013 ⁵⁶	Pre-adipocytes and adipocytes in culture	Saquinavir decreased mitochondrial membrane potencial and intracellular ATP in adipocytes.

Mechanism of action of Nelfinavir in cancer

Figure 1. Simplified mechanisms of action of nelfinavir in cancer. ERS: Endoplasmic reticulum stress.

Figure 2. A more detailed view of nelfinavir's action on ER stress.

Figure 3. Nelfinavir inhibition of SREBP1 as a cause of endoplasmic reticulum stress (ERS). For this figure the model of nelfinavir in liposarcoma was used.

Figure 4. SREBP is synthesized as an ER transmembrane protein and transported to the Golgi upon appropriate stimulus. For activation of SREBP it is necessary that luminal S1P (a protease) cleaves first, followed by intramembrane S2P (another protease) to liberate the transcriptionally active amino-terminal segments of nSREBP. NFV inhibits S1P and S2P, so that transcriptionally active SREBP is not produced. Accumulation of inactive SREBP is one of the UPR initiators.

Table 2. Nelfinavir anti-cancer activity in different tumor tissues*.

Tumor	Reference	Level
Hepatocarcinoma	Sun, 2014 ⁶²	Cell culture
Diffuse B cell lymphoma	Petrich, 2012 ⁶³	Cell culture
Glioblastoma	Kast, 2012 ⁶⁴	Cell culture
Liposarcoma	Pan, 2012 ⁶⁵	Clinical Trial (Phase I)
HER 2 positive, breast cancer cells	Shim, 2012 ⁶⁶	Cell culture
Acute myeloid leukemia	Kraus, 2014 ⁶⁷	Cell culture
Acute myeloid leukemia	Kraus, 2013 ⁶⁸	Cell culture
Cancer stem cells expressing embryonic genes**	Darini, 2013 ⁶⁹	Cell culture
Glioblastoma	Kas, 2013 ⁷⁰	Clinical
Castration resistant prostate cancer	Mathur, 2014 ⁷¹	Cell culture
Medullary thyroid cancer	Kushchayeva, Jensen, Recupero 2014 ⁷²	Cell culture
Medullary thyroid cancer	Kushchayeva, Jensen, Burman, 2014 ⁷³	Cell culture
Glioblastoma	Alonso-Basanta, 2014 ⁷⁴	Clinical Trial, Phase I
Rectal cancer	Buijsen J, 2013 ⁷⁵	Clinical Trial, Phase I
Refractory adenoid cystic carcinoma	Hoover, 2014 ⁷⁶	Clinical Trial, Phase II

*Saquinavir-NO has been tested in human melanoma cells with good results⁷⁷.

**It is necessary to underscore the finding that cancer stem cells expressing embryonic genes like Oct4, Sox2 and others, are particularly prone to apoptosis when PIs are used, particularly iopinavir (nelfinavir and saquinavir are also effective in this matter).

Figure 5. Summary of NFV anti-cancer mechanisms.

Table 3. Summary of clinical trials performed with NFV as an anti-cancer drug.

PROTOCOL	CHARACTERISTICS
NCT 0145106	SOLID TUMORS: A phase I trial of nelfinavir (Viracept) in adults with solid tumors
NCT 00436735	SOLID TUMORS: Nelfinavir in treating patients with metastatic, refractory, or recurrent solid tumors
NCT 02080416	Non-Hodgkin Lymphoma, Hodgkin Lymphoma, Kaposi Sarcoma, Gastric Cancer, Nasopharyngeal Cancer, EBV, Castleman Disease: nelfinavir for the treatment of gammaherpesvirus-related tumors
NCT 00589056	NSCLC: NFV, radiation therapy, cisplatin, and etoposide in treating patients with stage III non-small cell lung cancer that cannot be removed by surgery
NCT 01068327	Pancreatic cancer: stereotactic radiation therapy, nelfinavir mesylate, gemcitabine hydrochloride, leucovorin calcium, and fluorouracil in treating patients with locally advanced pancreatic cancer
NCT 01925378	Cervical intraepithelial neoplasia: a phase II single-arm intervention trial of nelfinavir in patients with grade 2/3 or 3 cervical intraepithelial neoplasia
NCT 01959672	Pancreatic cancer: nelfinavir mesylate in treating patients with locally advanced pancreatic cancer
NCT 01079286	Renal cell cancer: study of nelfinavir and temsirolimus in patients with advanced cancers
NCT 00704600	Rectal cancer: nelfinavir, a phase I/phase II rectal cancer study
NCT 01065844	Adenoid cystic cancer: nelfinavir in recurrent adenoid cystic cancer of the head and neck
NCT 01164709	Hematologic cancer: nelfinavir mesylate and bortezomib in treating patients with relapsed or progressive advanced hematologic cancer
NCT 01086332	Pancreatic cancer: evaluation of nelfinavir and chemoradiation for pancreatic cancer
NCT 01485731	Cervical cancer: safety study of nelfinavir + cisplatin + pelvic radiation therapy to treat cervical cancer
NCT 02024009	Pancreatic cancer: systemic therapy and chemoradiation in advanced localised pancreatic cancer - 2
NCT 00915694	Glioblastoma: nelfinavir, radiation therapy, and temozolomide in treating patients with glioblastoma multiforme
NCT 01108666	NSCLC: proton beam radiation with concurrent chemotherapy and nelfinavir for inoperable stage III non small cell lung cancer (NSCLC)
NCT 00791336	Study to evaluate using nelfinavir with chemoradiation for NSCLC
NCT 02207439	Larynx carcinoma: A phase II of nelfinavir, given with definitive, concurrent chemoradiotherapy (CTRT) in patients with locally-advanced, human papilloma virus (HPV) negative, squamous cell carcinoma larynx
NCT 01020292	Glioma: nelfinavir and concurrent radiation and temozolomide in patients with WHO grade IV glioma
NCT 01555281	Myeloma: nelfinavir and lenalidomide/dexamethasone in patients with progressive multiple myeloma that have failed lenalidomide-containing therapy
NCT 01728779	Metastatic lesions of the lung, liver or bone: stereotactic body radiation with nelfinavir for oligometastases.
NCT 00233948	Liposarcoma: nelfinavir mesylate in treating patients with recurrent, metastatic, or unresectable liposarcoma
NCT 02188537	Myeloma: nelfinavir as bortezomib-sensitizing drug in patients with proteasome inhibitor-nonresponsive myeloma
EudraCT Number: 2008-006302-42 University of Oxford	Pancreatic cancer: a phase II study in patients with locally advanced pancreatic carcinoma: ARC-II – Akt-inhibition by nelfinavir plus chemoradiation with gemcitabine and cisplatin

Interactions of NFV and PIs with other drugs

Indinavir and NFV increase anti-malarial action of artemisinin *in vitro* on *Plasmodium falciparum*⁷⁸, but artemisinin has also an off target anti-cancer activity. Thus it is reasonable to raise the question: may the association of artesiminin with NFV increase anti-cancer activity?

Another research team⁷⁰ has included both, NFV and artesiminin, in a multidrug repurposed protocol (CUSP 9) for the treatment of relapsed glioblastoma.

Celecoxib is an ER stressor that may enhance NFV anti-tumor activity⁷⁹.

Chloroquine and hydroxychloroquine are autophagy inhibitors and may work synergistically with NFV, downregulating autophagy and increasing apoptosis^{70,80}.

Nelfinavir may produce overproduction of mcl1 through upregulation of Erk 1/2, which would reduce apoptosis. The problem can be solved adding sorafenib^{81,82}.

In breast cancer cells, tamoxifen enhances anti-cancer activity of NFV⁸³. This synergism was independent of the estrogen receptor status so that the authors consider that the association of NFV and tamoxifen may be advantageous even in patients with no hormone responsive tumors.

Saquinavir has an interesting off target effect: it decreases intracellular ATP in adipocytes⁵⁶. If this effect is similar in tumor cells, an association with metformin and 2-deoxyglucose may produce anti-cancer activity⁸⁴⁻⁸⁶. There is growing interest on metabolic perturbators in cancer therapy and saquinavir may play a role in this field.

Clinical trials

A phase I dose escalation trial performed in 2014⁸⁷ established a MTD (maximum tolerated dose) of 3125 mg twice daily and described that 45% of patients with solid tumors treated with this dose decreased AKT activity and increased ERS indicators. This indicated a possible benefit in neuroendocrine tumor patients and also established that dose limiting toxicity consists in neutropenia.

The dose (3125 mg bid) is more than twice the dose used in HIV treatment. But lower doses, in the range of those used in HIV treatment have been tested, combining nelfinavir with chemoradiotherapy in pancreatic cancer with evidence of efficacy⁸⁸. No control group was used in this research, so comparison was established with known data from previous publications, mainly the favourable possibility of tumor resection after treatment.

Even lower doses (625 mg and 1250 mg bid) were tested in a phase I trial of NSCLC in stages IIIA/IIIB combined with chemoradiotherapy⁸⁹. In nine out of 12 patients a PET scan was available post-treatment with 100% overall response (56% complete response and 44% partial response). Unfortunately, in this trial there was no control group; 50% of the patients (six out of 12) lived for more than 22 months after treatment; 25% (three out of 12) lived without disease for more than 32 months. The results may be considered favourable, even without a control group.

Buijsen *et al.*⁷⁵ recommend a dose of 750 mg NFV bid for a phase II trial of this drug in combination with chemoradiotherapy in locally advanced rectal cancer.

Ongoing phase II trials are mainly in myeloma (associated with bortezomib or lenalomide), glioblastoma patients (associated with chemoradiation), pancreas (associated with gemcitabine and radiation) and lung. (See <http://www.clinicaltrials.gov>).

The first results of clinical trials did not show a meaningful improvement in the outcome of patients with refractory adenoid cystic carcinoma which is a malignant salivary gland tumor which usually has a poor prognosis. In this case NFV was used as monotherapy⁷⁶.

Why nelfinavir has a role to play in cancer therapy?

Akt activation is an important step in cancer phenotype and is a key player in acquisition and maintenance of cancer hallmarks. Akt is a nodal regulator of cellular survival pathways⁹⁰. There are no drugs at the present time that can inhibit this protein with a good safety profile. Wortmannin, perifosine and other chemicals designed for PI3K/Akt inhibition were too toxic for clinical use or have shown disappointing results, so they did not enter the medical practice. Insulin stimulation of Akt phosphorylation was reduced by 55% at achievable doses²⁰. At the same time there is clear evidence that it favours apoptosis and growth inhibition at clinically tolerable and achievable doses.

This anti-Akt activity of NFV can be reinforced by concomitant mTOR inhibition which results in synergistic cytotoxicity⁶³. This may be due to the fact that mTOR inhibition without Akt inhibition eliminates a negative biofeedback loop on Akt, producing increased phosphorylation of Akt⁹¹. According to Sarbassov⁹² this negative feedback is born in the mTORC2 complex.

According to Carracedo⁹³, this negative feedback loop goes as far as PI3K (Figure 6).

mTOR inhibitors have become a new and important tool against cancer, for example in renal cell carcinoma. But the negative biofeedback loop on Akt must be solved to achieve really good results. NFV could be the clue.

But the most important anti-tumor activity of NVR is not limited to Akt inhibition but ER stress and UPR which may be one of the pathways leading to apoptosis⁹⁴.

Additional features of NFV and other protease inhibitors are

- 1) the ability to sensitize cancer cells to chemoradiotherapy,
- 2) anti-angiogenesis by decreasing VEGF/HIF expression,

Figure 6. Negative feedback loop.

Figure 7. Mechanism of synergy between NFV and mTOR inhibitors. At the left is depicted the pathway under normal or pathological circumstances; in the middle drawing mTOR inhibition is counterbalanced by Akt activation due to loss of the negative biofeedback circuit; on the right, inhibition of both, mTOR and Akt may result in increased anti-cancer results.

- 3) decreased expression of FAS (fatty acid synthase),
- 4) the combination of radiation and PIs is well tolerated^{39,94},
- 5) NFV cancer cell killing ability can easily be enhanced with other ER stressors like celecoxib⁷⁹. Cho *et al.* found enhanced killing of chemoresistant breast cancer cells after celecoxib treatment that aggravated ER stress; perillyl alcohol is another stress aggravator that has been used with that purpose⁹⁶,
- 6) In head and neck cancer related to HPV, NFV produced down-regulation of Akt and radiosensitization⁹⁷,
- 7) NFV not only down-regulates Akt but also MAPK (in adenoid cystic cancer)⁹⁸, and retards oral cell proliferation including normal keratinocytes and squamous cell cancer⁹⁹,
- 8) There are evidences, at least in pancreatic cancer, that NFV dependent down-regulation of Akt is independent of the mutational status of K-ras¹⁰⁰,
- 9) There is clear evidence (in glioblastoma) of the relation between NFV and apoptosis through the following pathway⁴⁶:

NFV-----ER stress-----CHOP-----up regulation of TRAIL receptor DR5

- 10) Down-regulation of MMP-9 (reduced expression and secretion of MMP-9 by human preadipocytes)^{64,101},
- 11) Increased apoptosis by NFV when associated with anti-autophagy drugs like chloroquine or hydroxychloroquines, particularly in triple negative breast cancer cells¹⁰².

Possible controversies

The SREBP pathway for regulation of fat metabolism is initiated through proteolytic cleavage of precursor forms of the SREBPs (125 Kd protein) in ER membranes. When cells are in need of

sterol, the precursor SREBPs are hydrolyzed by a 2-step mechanism involving membrane-bound serine protease S1P and a metalloprotease S2P. The N-terminal fragment of SREBP (nSREBP) is a 68 Kd protein that translocates to the nucleus where it works as a promoter-enhancer, binding to sterol regulatory elements located in DNA and activates gene transcription (Figure 4). The nuclear SREBP can be rapidly degraded by a proteasome-mediated mechanism. This provides regulation of gene transcriptional activities¹⁰³.

Transgenic mice over-expressing the constitutively active nuclear forms of the SREBPs (nSREBPs) revealed that overexpression of SREBP-1 or SREBP-2 leads to activation of genes involved in the cholesterol and fatty acid biosynthesis cascades. These transgenic mice displayed the classical features of generalized lipodystrophy, similar to those found in patients under PI therapy¹⁰⁴.

Riddle *et al.* in 2001¹⁰⁵ found that PI therapy (they used ritonavir) induced the accumulation of activated SREBP-1 and SREBP-2 in the nucleus of liver and adipose tissues. As a consequence, fatty acid and cholesterol biosynthesis were increased in these tissues. The authors consider that lipodystrophy, hyperlipidemia, and insulin resistance, are the consequence of activated SREBP-1 and SREBP-2 accumulation in the nucleus of liver and adipose tissues. The possible mechanism for these events, according to their criteria is PI suppression of activated SREBP degradation in the nucleus. In summary, Riddle's study showed that ritonavir induced lipid metabolism abnormalities through stabilization of activated SREBP-1 and SREBP-2 in the nucleus of liver and adipose tissues.

These findings are in contrast with those of Guan^{49,106} where NFV inhibited the nuclear translocation of the sterol regulatory element binding protein-1 (SREBP-1) in castration resistant prostate cancer and liposarcoma through inhibition of S1P. This led to accumulation of unprocessed SREBP-1.

Riddle *et al.* described accumulation of processed SREBP-1 in the liver and adipose tissue while Guan found accumulation of unprocessed SREBP1 in ER and Golgi with no translocation to nucleus in liposarcoma and castration resistant prostate cancer tissue.

The controversy may be explained in the following way:

- 1) There are three different isoforms of SREBP: SREBP-1a, SREBP-1c and SREBP-2.
- 2) SREBP-1a and -1c have different expression profiles: SREBP-1a is highly expressed in proliferating cells, such as cancer cells, while SREBP-1c is the predominant form in normal cells, particularly hepatocytes¹⁰⁴.
- 3) The target genes for the three SREBP isoforms are different.
- 4) Riddle *et al.* found increased SREBP-1 and two in the nucleus of liver and adipose tissues; these SREBPs are the active form (they make no difference between SREBP-1a and SREBP-1c).
- 5) Guan *et al.* found increased SREBP in Golgi in the inactive form (precursor) of tumor tissues treated with NFV.
- 6) It is possible that tumor tissues that overexpress SREBP-1a behave in a different way than liver and adipose tissue that overexpress SREBP-1c.
- 7) Riddle *et al.* tested ritonavir and Guan *et al.* tested NFV, so the pharmacological effects between these PIs may differ.

A second controversy that stems from the one described above is on the effect of NFV on FAS:

- 1) According to Guan *et al.*¹⁰⁶, NFV decreases expression of FAS in liposarcoma cells and castrate resistant prostate cancer as was depicted in Figure 3.
- 2) According to Lenhard *et al.* 2000¹⁰⁷, NFV increases expression of FAS in HepG2 cells (which show many of the normal biochemical functions of non tumor liver parenchymal cells).

May this difference be due to tissue-specific effects of NFV? Does NFV have different effects in tumor tissues and normal tissues?

To definitely solve these controversies, it is necessary to proceed with further experimental research, but the findings described above necessarily raise the doubt that mechanisms that work in tumor cells might be slightly different from those working in hepatocytes and adipocytes.

Possible negative aspects of PIs in cancer

Despite the anti-cancer activity of NFV and PIs, these drugs do not reduce the risk of developing cancer in HIV population¹⁰⁸ and also exert certain depression of immunological functions, interfering with the differentiation program of monocytes into dendritic cells¹⁰⁹.

PIs increase the expression of P-glycoprotein (ABCB1) in Kaposi's sarcoma cell lines increasing the multidrug resistance phenotype¹¹⁰.

At the same time ABCB1 expression depends on Akt activation¹¹¹ and NFV inhibits partially Akt. The final result of the two antagonistic aspects requires further research.

There are well known undesirable side effects with HIV PIs, like hyperlipidemia, insulin resistance and lipodystrophy (peripheral fat wasting and excessive central fat deposition). One of the main responsible mechanisms of these side effects is the suppression of the breakdown of SREBP in the liver and adipose tissues resulting in increased fatty acid and cholesterol biosynthesis. SREBP accumulation in adipose tissue causes lipodystrophy.

PIs suppress proteasome-mediated breakdown of nascent apolipoprotein (apo) B, resulting in the overproduction of triglyceride. Finally, PIs also suppress the inhibition of the glucose transporter GLUT-4 activity in adipose tissue and muscle. This contributes directly to insulin resistance and diabetes¹¹².

Hepatomegaly and hepatic steatosis are direct consequences of the metabolic alterations explained above¹⁰⁵.

New PIs with anti-cancer activity

In 2010 You *et al.*¹¹³ synthesized a new indinavir analogue with remarkable anti-cancer activity, similar to NFV: CH05-10. This drug achieved similar cytotoxicity to NFV but at lower concentrations, against leukaemia, melanoma, ovarian and prostate cancer cell lines.

In 2009 Saquinavir-NO was introduced¹¹⁴; it showed interesting anti-cancer properties in melanoma xenografts with significantly lower toxicity than saquinavir.

Conclusions

The most relevant mechanisms of PIs anti-cancer activity are Akt inhibition and ER stress.

Following our exhaustive analysis of the current medical literature we conclude that NFV anti-cancer activity is mainly dependent on ER stress-UPR.

Akt inhibition plays also a very important role but is not the unique or main source of anti-cancer effects.

The evidences that support these conclusions are:

- 1) Even at very high doses of NFV (3,125 mg bid), Akt achieved a level of inhibition around 55% in cell culture²⁰.
- 2) NFV is at the same time a strong ER stressor and an Akt inhibitor.
- 3) Anti-cancer activity can be achieved at much lower doses than those necessary for Akt inhibition.
- 4) Increasing ER stress by adding Celecoxib to NFV enhances cytotoxicity.
- 5) Autophagy, which is one of the mechanisms cells use to survive increasing ER stress¹¹⁵, is inhibited by adding chloroquine or hydroxychloroquine to NFV. In this case, apoptosis is significantly enhanced^{80,102}.

- 6) The PIs with anti-cancer activity like NFV, ritonavir¹¹⁶, saquinavir¹¹⁷, and the experimental drug CH05-10¹¹³ are strong ER stressors. Amprenavir is a PI that induces no ER stress and its anti-cancer activity is significantly weaker than that of NFV, although it has Akt inhibiting effects.
- 7) Ritonavir, which is ER stressor, shows anti-cancer activity although it does not down-regulate Akt at concentrations usually found in HIV patients²³.
- 8) Inhibition of proteasome with bortezomib has a synergistic effect with NFV apoptotic activity¹¹⁸
- 9) PIs can be classified regarding anti-cancer activity at clinically achievable concentration in patients in
 - A) Akt inhibitors only: e.g. amprenavir
 - B) ER stressors only: e.g. ritonavir
 - C) Akt inhibitors and ER stressors: examples NFV and experimental PI CH05-10. Of course this is the group that shows stronger anti-cancer activity.

There is enough evidence of NFV anti-cancer effects and there is adequate knowledge of how this activity works, so that NFV deserves well designed phase II clinical trials, as adjunct cancer therapy.

Associations with proteasomal inhibitors, celecoxib and other cell stressor should also be investigated in the clinical setting due to possible synergy. Tamoxifen with NFV may show interesting results in breast cancer.

Although a large amount of publications, including reviews, have been written on NFV and other PIs in cancer, none has been dedicated to a thorough examination and analysis of the mode of action of these pharmaceuticals as off target drugs (with the exception of the review by Gantt *et al.*²). It is hoped that this review will encourage an increment adequately powered and well-designed clinical trials in the various cancer types, beyond the phase I trials that have been recently performed, and specifically trials where these compounds may be tested in association with other known anti-cancer

pharmaceuticals like NFV associated to bortezomib and hydroxychloroquine in myeloma, or mTOR inhibitors with NFV in HNSCC and many other possible combinations where the dual feature of NFV, ER stressor and Akt inhibitor, are required.

In myeloma NFV increases proteasome inhibition by bortezomib and may overcome resistance to proteasomal inhibitors. This is an action exclusive of NFV and not shared with other PIs⁶⁷, with the additional advantage that NFV shows the highest cytotoxic activity against primary myeloma cells.

If apoptosis is described as a cascade, then apoptosis stimulator drugs like NFV should be viewed as enhancers of this cascade. An initiator of the cascade is still necessary, for example chemoradiotherapy. After this initial step, apoptosis stimulator drugs increase the amount of cells entering this pathway. This might be one of possible reasons why nelfinavir alone has shown poor results in a clinical trial used as monotherapy.

This does not mean that NFV cannot act as an initiator, but the evidences show that it is prone to be an enhancer of apoptosis rather than an initiator.

Future directions

All the evidences presented in this review reinforce the concept that NFV is a useful drug in cancer treatment. It should be considered in association with chemoradiotherapy in the design of new protocols for diseases like multiple myeloma (in association with bortezomib and hydroxychloroquine) and prostate, pancreas and lung cancer where clinical trials are ongoing. New PIs are being developed with better anti-cancer profile like CH05-10 and saquinavir-NO⁷⁷ and further development of new PIs with stronger anti-cancer activity, will probably go on in the future.

Competing interests

No competing interests were disclosed.

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References

1. Pai VB, Nahata MC: **Nelfinavir mesylate: a protease inhibitor.** *Ann Pharmacother.* 1999; **33**(3): 325–339.
[PubMed Abstract](#) | [Publisher Full Text](#)
2. Gantt S, Casper C, Ambinder RF: **Insights into the broad cellular effects of nelfinavir and the HIV protease inhibitors supporting their role in cancer treatment and prevention.** *Curr Opin Oncol.* 2013; **25**(5): 495–502.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
3. Flexner C: **HIV-protease inhibitors.** *N Engl J Med.* 1998; **338**(18): 1281–1292.
[PubMed Abstract](#) | [Publisher Full Text](#)
4. Volberding PA, Deeks SG: **Antiretroviral therapy for HIV infection: promises and problems.** *JAMA.* 1998; **279**(17): 1343–1344.
[PubMed Abstract](#) | [Publisher Full Text](#)
5. Zhang KE, Wu E, Patick AK, *et al.*: **Circulating metabolites of the human immunodeficiency virus protease inhibitor nelfinavir in humans: structural identification, levels in plasma, and antiviral activities.** *Antimicrob Agents Chemother.* 2001; **45**(4): 1086–1093.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
6. Chow WA, Jiang C, Guan M: **Anti-HIV drugs for cancer therapeutics: back to the future?** *Lancet Oncol.* 2009; **10**(1): 61–71.
[PubMed Abstract](#) | [Publisher Full Text](#)
7. Krischer J, Rutschmann O, Hirschel B, *et al.*: **Regression of Kaposi's sarcoma during therapy with HIV-1 protease inhibitors: a prospective pilot study.** *J Am Acad Dermatol.* 1998; **38**(4): 594–598.
[PubMed Abstract](#) | [Publisher Full Text](#)

8. Lebbé C, Blum L, Pellet C, *et al.*: **Clinical and biological impact of antiretroviral therapy with protease inhibitors on HIV-related Kaposi's sarcoma.** *AIDS*. 1998; 12(7): F45–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
9. Niehues T, Horneff G, Megahed M, *et al.*: **Complete regression of AIDS-related Kaposi's sarcoma in a child treated with highly active antiretroviral therapy.** *AIDS*. 1999; 13(9): 1148–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
10. Sgadari C, Monini P, Barillari G, *et al.*: **Use of HIV protease inhibitors to block Kaposi's sarcoma and tumour growth.** *Lancet Oncol*. 2003; 4(9): 537–47. Review.
[PubMed Abstract](#) | [Publisher Full Text](#)
11. Schmidtke G, Holzthutter HG, Bogoy M, *et al.*: **How an inhibitor of the HIV-1 protease modulates proteasome activity.** *J Biol Chem*. 1999; 274(50): 35734–40.
[PubMed Abstract](#) | [Publisher Full Text](#)
12. Ikezoe T, Daar ES, Hisatake J, *et al.*: **HIV-1 protease inhibitors decrease proliferation and induce differentiation of human myelocytic leukemia cells.** *Blood*. 2000; 96(10): 3553–9.
[PubMed Abstract](#)
13. Ikezoe T, Saito T, Bandobashi K, *et al.*: **HIV-1 protease inhibitor induces growth arrest and apoptosis of human multiple myeloma cells via inactivation of signal transducer and activator of transcription 3 and extracellular signal-regulated kinase 1/2.** *Mol Cancer Ther*. 2004; 3(4): 473–9.
[PubMed Abstract](#)
14. Andre P, Groettrup M, Klennerman P, *et al.*: **An inhibitor of HIV-1 protease modulates proteasome activity, antigen presentation, and T cell responses.** *Proc Natl Acad Sci U S A*. 1998; 95(22): 13120–13124.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
15. Gaedicke S, Firat-Geier E, Constantiniu O, *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*. 2002; 62(23): 6901–8.
[PubMed Abstract](#)
16. Sgadari C, Barillari G, Toschi E, *et al.*: **HIV protease inhibitors are potent anti-angiogenic molecules and promote regression of Kaposi sarcoma.** *Nat Med*. 2002; 8(3): 225–32.
[PubMed Abstract](#) | [Publisher Full Text](#)
17. Pajonk F, Himmelsbach J, Riess K, *et al.*: **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*. 2002; 62(18): 5230–5.
[PubMed Abstract](#)
18. Pajonk F, McBride WH: **Ionizing radiation affects 26S proteasome function and associated molecular responses, even at low doses.** *Radiother Oncol*. 2001; 59(2): 203–212.
[PubMed Abstract](#) | [Publisher Full Text](#)
19. Olson DP, Scadden DT, D'Aquila RT, *et al.*: **The protease inhibitor ritonavir inhibits the functional activity of the multidrug resistance related-protein 1 (MRP-1).** *AIDS*. 2002; 16(13): 1743–1747.
[PubMed Abstract](#) | [Publisher Full Text](#)
20. Zhou JQ, Xiang Z, Schutt M: **[Impairment of IRS-2 signaling in rat insulinoma INS-1 cells by nelfinavir].** *Zhejiang Da Xue Xue Bao Yi Xue Ban*. 2004; 33(4): 311–4. Chinese.
[PubMed Abstract](#)
21. Gupta A, Zhang Y, Unadkat JD, *et al.*: **HIV protease inhibitors are inhibitors but not substrates of the human breast cancer resistance protein (BCRP/ABCG2).** *J Pharmacol Exp Ther*. 2004; 310(1): 334–341.
[PubMed Abstract](#) | [Publisher Full Text](#)
22. Piccinini M, Rinaudo MT, Anselmino A, *et al.*: **The HIV protease inhibitors nelfinavir and saquinavir, but not a variety of HIV reverse transcriptase inhibitors, adversely affect human proteasome function.** *Antivir Ther*. 2005; 10(2): 215–23.
[PubMed Abstract](#)
23. Gupta AK, Cerniglia GJ, Mick R, *et al.*: **HIV protease inhibitors block Akt signaling and radiosensitize tumor cells both *in vitro* and *in vivo*.** *Cancer Res*. 2005; 65(18): 8256–65.
[PubMed Abstract](#) | [Publisher Full Text](#)
24. Yang Y, Ikezoe T, Takeuchi T, *et al.*: **HIV-1 protease inhibitor induces growth arrest and apoptosis of human prostate cancer LNCaP cells *in vitro* and *in vivo* in conjunction with blockade of androgen receptor STAT3 and AKT signaling.** *Cancer Sci*. 2005; 96(7): 425–33.
[PubMed Abstract](#) | [Publisher Full Text](#)
25. Yang Y, Ikezoe T, Nishioka C, *et al.*: **NFV, an HIV-1 protease inhibitor, induces growth arrest, reduced Akt signalling, apoptosis and docetaxel sensitisation in NSCLC cell lines.** *Br J Cancer*. 2006; 95(12): 1653–1662.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
26. Chow WA, Guo S, Valdes-Albini F: **Nelfinavir induces liposarcoma apoptosis and cell cycle arrest by upregulating sterol regulatory element binding protein-1.** *Anticancer Drugs*. 2006; 17(8): 891–903.
[PubMed Abstract](#) | [Publisher Full Text](#)
27. Pore N, Gupta AK, Cerniglia GJ, *et al.*: **HIV protease inhibitors decrease VEGF/HIF-1 α expression and angiogenesis in glioblastoma cells.** *Neoplasia*. 2006; 8(11): 889–95.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
28. Pore N, Gupta AK, Cerniglia GJ, *et al.*: **Nelfinavir down-regulates hypoxia-inducible factor 1 α and VEGF expression and increases tumor oxygenation: implications for radiotherapy.** *Cancer Res*. 2006; 66(18): 9252–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
29. Hampson L, Kitchener HC, Hampson IN: **Specific HIV protease inhibitors inhibit the ability of HPV16 E6 to degrade p53 and selectively kill E6-dependent cervical carcinoma cells *in vitro*.** *Antivir Ther*. 2006; 11(6): 813–25.
[PubMed Abstract](#)
30. Ben-Romano R, Rudich A, Etzion S, *et al.*: **Nelfinavir induces adipocyte insulin resistance through the induction of oxidative stress: differential protective effect of antioxidant agents.** *Antivir Ther*. 2006; 11(8): 1051–1060.
[PubMed Abstract](#)
31. Gupta V, Samuleson CG, Su S, *et al.*: **Nelfinavir potentiation of imatinib cytotoxicity in meningioma cells via survivin inhibition.** *Neurosurg Focus*. 2007; 23(4): E9.
[PubMed Abstract](#) | [Publisher Full Text](#)
32. Jiang W, Mikochik PJ, Ra JH, *et al.*: **HIV protease inhibitor nelfinavir inhibits growth of human melanoma cells by induction of cell cycle arrest.** *Cancer Res*. 2007; 67(3): 1221–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
33. Jiang Z, Pore N, Cerniglia GJ, *et al.*: **Phosphatase and tensin homologue deficiency in glioblastoma confers resistance to radiation and temozolomide that is reversed by the protease inhibitor nelfinavir.** *Cancer Res*. 2007; 67(9): 4467–73.
[PubMed Abstract](#) | [Publisher Full Text](#)
34. Gills JJ, Lopiccolo J, Tsurutani J: **Nelfinavir, A lead HIV protease inhibitor, is a broad-spectrum, anticancer agent that induces endoplasmic reticulum stress, autophagy, and apoptosis *in vitro* and *in vivo*.** *Clin Cancer Res*. 2007; 13(17): 5183–94.
[PubMed Abstract](#) | [Publisher Full Text](#)
35. Cuneo KC, Tu T, Geng L, *et al.*: **HIV protease inhibitors enhance the efficacy of irradiation.** *Cancer Res*. 2007; 67(10): 4886–93.
[PubMed Abstract](#) | [Publisher Full Text](#)
36. Pyrko P, Kardosh A, Wang W, *et al.*: **HIV-1 protease inhibitors nelfinavir and atazanavir induce malignant glioma death by triggering endoplasmic reticulum stress.** *Cancer Res*. 2007; 67(22): 10920–8.
[PubMed Abstract](#) | [Publisher Full Text](#)
37. De Barros S, Zakaroff-Girard A, Lafontan M, *et al.*: **Inhibition of human preadipocyte proteasomal activity by HIV protease inhibitors or specific inhibitor lactacystin leads to a defect in adipogenesis, which involves matrix metalloproteinase-9.** *J Pharmacol Exp Ther*. 2007; 320(1): 291–299.
[PubMed Abstract](#) | [Publisher Full Text](#)
38. Gills JJ, Lopiccolo J, Dennis PA: **Nelfinavir, a new anti-cancer drug with pleiotropic effects and many paths to autophagy.** *Autophagy*. 2008; 4(1): 107–109.
[PubMed Abstract](#) | [Publisher Full Text](#)
39. Plastaras JP, Vapiwala N, Ahmed MS, *et al.*: **Validation and toxicity of PI3K/Akt pathway inhibition by HIV protease inhibitors in humans.** *Cancer Biol Ther*. 2008; 7(5): 628–635.
[PubMed Abstract](#) | [Publisher Full Text](#)
40. Brünig A, Vogel M, Burger P, *et al.*: **Nelfinavir induces TRAIL receptor upregulation in ovarian cancer cells.** *Biochem Biophys Res Commun*. 2008; 377(4): 1309–1314.
[PubMed Abstract](#) | [Publisher Full Text](#)
41. Giri N, Agarwal S, Shaik N, *et al.*: **Substrate-dependent breast cancer resistance protein (Bcrp1/Abcg2)-mediated interactions: consideration of multiple binding sites in *in vitro* assay design.** *Drug Metab Dispos*. 2009; 37(3): 560–70.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
42. Brünig A, Burger P, Vogel M, *et al.*: **Nelfinavir induces the unfolded protein response in ovarian cancer cells, resulting in ER vacuolization, cell cycle retardation and apoptosis.** *Cancer Biol Ther*. 2009; 8(3): 226–32.
[PubMed Abstract](#) | [Publisher Full Text](#)
43. Dewan MZ, Tomita M, Katano H, *et al.*: **An HIV protease inhibitor, ritonavir targets the nuclear factor-kappaB and inhibits the tumor growth and infiltration of EBV-positive lymphoblastoid B cells.** *Int J Cancer*. 2009; 124(3): 622–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
44. Wang P, Kelly C, Harvey A, *et al.*: **Quantitative analysis of tumor vascular structure after drug treatment.** *Conf Proc IEEE Eng Med Biol Soc*. 2010; 2010: 726–729.
[PubMed Abstract](#) | [Publisher Full Text](#)
45. Xie L, Evangelidis T, Xie L, *et al.*: **Drug discovery using chemical systems biology: weak inhibition of multiple kinases may contribute to the anti-cancer effect of nelfinavir.** *PLoS Comput Biol*. 2011; 7(4): e1002037.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
46. Tian X, Ye J, Alonso-Basanta M, *et al.*: **Modulation of CCAAT/enhancer binding protein homologous protein (CHOP)-dependent DR5 expression by nelfinavir sensitizes glioblastoma multiforme cells to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL).** *J Biol Chem*. 2011; 286(33): 29408–16.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
47. Brünig A, Vogel M, Mylonas I, *et al.*: **Bortezomib targets the caspase-like proteasome activity in cervical cancer cells, triggering apoptosis that can be enhanced by nelfinavir.** *Curr Cancer Drug Targets*. 2011; 11(7): 799–809.
[PubMed Abstract](#) | [Publisher Full Text](#)

48. Zeng J, See AP, Aziz K, *et al.*: Nelfinavir induces radiation sensitization in pituitary adenoma cells. *Cancer Biol Ther.* 2011; 12(7): 657–63.
[PubMed Abstract](#) | [Publisher Full Text](#)
49. Guan M, Fousek K, Chow WA: Nelfinavir inhibits regulated intramembrane proteolysis of sterol regulatory element binding protein-1 and activating transcription factor 6 in castration-resistant prostate cancer. *FEBS J.* 2012; 279(13): 2399–411.
[PubMed Abstract](#) | [Publisher Full Text](#)
50. Bono C, Karlin L, Harel S, *et al.*: The human immunodeficiency virus-1 protease inhibitor nelfinavir impairs proteasome activity and inhibits the proliferation of multiple myeloma cells *in vitro* and *in vivo*. *Haematologica.* 2012; 97(7): 1101–1109.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
51. Brüning A, Matsingou C, Brem GJ, *et al.*: Inhibin beta E is upregulated by drug-induced endoplasmic reticulum stress as a transcriptional target gene of ATF4. *Toxicol Appl Pharmacol.* 2012; 264(2): 300–304.
[PubMed Abstract](#) | [Publisher Full Text](#)
52. Barillari G, Iovane A, Bacigalupo I, *et al.*: Ritonavir or saquinavir impairs the invasion of cervical intraepithelial neoplasia cells via a reduction of MMP expression and activity. *AIDS.* 2012; 26(8): 909–919.
[PubMed Abstract](#) | [Publisher Full Text](#)
53. Timeus F, Crescenzo N, Doria A, *et al.*: *in vitro* anti-neuroblastoma activity of saquinavir and its association with imatinib. *Oncol Rep.* 2012; 27(3): 734–740.
[PubMed Abstract](#) | [Publisher Full Text](#)
54. Ismail WI, King JA, Anwar K, *et al.*: Indinavir and nelfinavir inhibit proximal insulin receptor signaling and salicylate abrogates inhibition: potential role of the NFkappa B pathway. *J Cell Biochem.* 2013; 114(8): 1729–37.
[PubMed Abstract](#) | [Publisher Full Text](#)
55. Escalante AM, McGrath RT, Karolak MR, *et al.*: Preventing the autophagic survival response by inhibition of calpain enhances the cytotoxic activity of bortezomib *in vitro* and *in vivo*. *Cancer Chemother Pharmacol.* 2013; 71(6): 1567–76.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
56. Bociaga-Jasik M, Polus A, Goralska J, *et al.*: Metabolic effects of the HIV protease inhibitor–saquinavir in differentiating human preadipocytes. *Pharmacol Rep.* 2013; 65(4): 937–950.
[PubMed Abstract](#) | [Publisher Full Text](#)
57. Cox JS, Walter P: A novel mechanism for regulating activity of a transcription factor that controls the unfolded protein response. *Cell.* 1996; 87(3): 391–404.
[PubMed Abstract](#) | [Publisher Full Text](#)
58. Tsai YC, Weissman AM: The Unfolded Protein Response, Degradation from Endoplasmic Reticulum and Cancer. *Genes Cancer.* 2010; 1(7): 764–778.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
59. Mori K, Ogawa N, Kawahara T, *et al.*: mRNA splicing-mediated C-terminal replacement of transcription factor Hac1p is required for efficient activation of the unfolded protein response. *Proc Natl Acad Sci U S A.* 2000; 97(9): 4660–4665.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
60. Schönthal AH: Endoplasmic reticulum stress: its role in disease and novel prospects for therapy. *Scientifica (Cairo).* 2012; 2012: 857516. Review.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
61. Simmons SO, Fan CY, Ramabhadran R: Cellular stress response pathway system as a sentinel ensemble in toxicological screening. *Toxicol Sci.* 2009; 111(2): 202–225. (Review).
[PubMed Abstract](#) | [Publisher Full Text](#)
62. Sun L, Niu L, Zhu X, *et al.*: Antitumour effects of a protease inhibitor, nelfinavir, in hepatocellular carcinoma cancer cells. *J Chemother.* 2012; 24(3): 161–6.
[PubMed Abstract](#) | [Publisher Full Text](#)
63. Petrich AM, Leshchenko V, Kuo PY, *et al.*: Akt inhibitors MK-2206 and nelfinavir overcome mTOR inhibitor resistance in diffuse large B-cell lymphoma. *Clin Cancer Res.* 2012; 18(9): 2534–44.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
64. Kast RE, Halatsch ME: Matrix metalloproteinase-2 and -9 in glioblastoma: a trio of old drugs-captopril, disulfiram and nelfinavir-are inhibitors with potential as adjunctive treatments in glioblastoma. *Arch Med Res.* 2012; 43(3): 243–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
65. Pan J, Mott M, Xi B, *et al.*: Phase I study of nelfinavir in liposarcoma. *Cancer Chemother Pharmacol.* 2012; 70(6): 791–799.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
66. Shim JS, Rao R, Beebe K, *et al.*: Selective inhibition of HER2-positive breast cancer cells by the HIV protease inhibitor nelfinavir. *J Natl Cancer Inst.* 2012; 104(20): 1576–90.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
67. Kraus M, Bader J, Overkleff H, *et al.*: Nelfinavir augments proteasome inhibition by bortezomib in myeloma cells and overcomes bortezomib and carfilzomib resistance. *Blood Cancer J.* 2013; 3: e103.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
68. Kraus M, Müller-Ide H, Rückrich T, *et al.*: Ritonavir, nelfinavir, saquinavir and lopinavir induce proteotoxic stress in acute myeloid leukemia cells and sensitize them for proteasome inhibitor treatment at low micromolar drug concentrations. *Leuk Res.* 2014; 38(3): 383–92.
[PubMed Abstract](#) | [Publisher Full Text](#)
69. Darini CY, Martin P, Azoulay S, *et al.*: Targeting cancer stem cells expressing an embryonic signature with anti-proteases to decrease their tumor potential. *Cell Death Dis.* 2013; 4: e706.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
70. Kast RE, Boockvar JA, Brüning A, *et al.*: A conceptually new treatment approach for relapsed glioblastoma: coordinated undermining of survival paths with nine repurposed drugs (CUSP9, by the International Initiative for Accelerated Improvement of Glioblastoma Care. *Oncotarget.* 2013; 4(4): 502–530.
[PubMed Abstract](#) | [Free Full Text](#)
71. Mathur A, Abd Elmageed ZY, Liu X, *et al.*: Subverting ER-stress towards apoptosis by nelfinavir and curcumin coexposure augments docetaxel efficacy in castration resistant prostate cancer cells. *PLoS One.* 2014; 9(8): e103109.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
72. Kushchayeva Y, Jensen K, Recupero A, *et al.*: The HIV protease inhibitor nelfinavir down-regulates RET signaling and induces apoptosis in medullary thyroid cancer cells. *J Clin Endocrinol Metab.* 2014; 99(5): E734–45.
[PubMed Abstract](#) | [Publisher Full Text](#)
73. Kushchayeva Y, Jensen K, Burman KD, *et al.*: Repositioning therapy for thyroid cancer: new insights on established medications. *Endocr Relat Cancer.* 2014; 21(3): R183–94.
[PubMed Abstract](#) | [Publisher Full Text](#)
74. Alonso-Basanta M, Fang P, Maity A, *et al.*: A phase I study of nelfinavir concurrent with temozolomide and radiotherapy in patients with glioblastoma multiforme. *J Neurooncol.* 2014; 116(2): 365–72.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
75. Buijsen J, Lammering G, Jansen RL, *et al.*: Phase I trial of the combination of the Akt inhibitor nelfinavir and chemoradiation for locally advanced rectal cancer. *Radiother Oncol.* 2013; 107(2): 184–188.
[PubMed Abstract](#) | [Publisher Full Text](#)
76. Hoover AC, Milhem MM, Anderson CM, *et al.*: Efficacy of nelfinavir as monotherapy in refractory adenoid cystic carcinoma: Results of a phase II clinical trial. *Head Neck.* 2014.
[PubMed Abstract](#) | [Publisher Full Text](#)
77. Donia M, Mangano K, Fagone P, *et al.*: Unique antineoplastic profile of Saquinavir-NO, a novel NO-derivative of the protease inhibitor Saquinavir, on the *in vitro* and *in vivo* tumor formation of A375 human melanoma cells. *Oncol Rep.* 2012; 28(2): 682–688.
[PubMed Abstract](#) | [Publisher Full Text](#)
78. Mishra LC, Bhattacharya A, Sharma M, *et al.*: HIV protease inhibitors, indinavir or nelfinavir, augment antimalarial action of artemisinin *in vitro*. *Am J Trop Med Hyg.* 2010; 82(1): 148–150.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
79. Cho HY, Thomas S, Golden EB, *et al.*: Enhanced killing of chemo-resistant breast cancer cells via controlled aggravation of ER stress. *Cancer Lett.* 2009; 282(1): 87–97.
[PubMed Abstract](#) | [Publisher Full Text](#)
80. Mahoney E, Maddocks K, Flynn J, *et al.*: Identification of endoplasmic reticulum stress-inducing agents by antagonizing autophagy: a new potential strategy for identification of anti-cancer therapeutics in B-cell malignancies. *Leuk Lymphoma.* 2013; 54(12): 2685–92.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
81. Brüning A, Burger P, Vogel M, *et al.*: Nelfinavir induces mitochondria protection by ERK 1/2-mediated mcl-1 stabilization that can be overcome by sorafenib. *Invest New Drugs.* 2010; 28(5): 535–42.
[PubMed Abstract](#) | [Publisher Full Text](#)
82. Brüning A, Rahmeh M, Ginkelmaier A, *et al.*: The mitochondria-independent cytotoxic effect of nelfinavir on leukemia cells can be enhanced by sorafenib-mediated mcl-1 downregulation and mitochondrial membrane destabilization. *Mol Cancer.* 2010; 9: 19.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
83. Brüning A, Friese K, Burges A, *et al.*: Tamoxifen enhances the cytotoxic effects of nelfinavir in breast cancer cells. *Breast Cancer Res.* 2010; 12(4): R45.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
84. Cheng G, Zielonka J, McAllister D, *et al.*: Profiling and targeting of cellular bioenergetics: inhibition of pancreatic cancer cell proliferation. *Br J Cancer.* 2014; 111(1): 85–93.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
85. Cheong JH, Park ES, Liang J, *et al.*: Dual inhibition of tumor energy pathway by 2-deoxyglucose and metformin is effective against a broad spectrum of preclinical cancer models. *Mol Cancer Ther.* 2011; 10(12): 2350–62.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
86. Ben Sahra I, Laurent K, Giuliano S, *et al.*: Targeting cancer cell metabolism: the combination of metformin and 2-deoxyglucose induces p53-dependent apoptosis in prostate cancer cells. *Cancer Res.* 2010; 70(6): 2465–75.
[PubMed Abstract](#) | [Publisher Full Text](#)
87. Blumenthal GM, Gills JJ, Ballas MS, *et al.*: A phase I trial of the HIV protease inhibitor nelfinavir in adults with solid tumors. *Oncotarget.* 2014; 5(18): 8161–72.
[PubMed Abstract](#) | [Free Full Text](#)
88. Brunner TB, Geiger M, Grabenbauer GG, *et al.*: Phase I trial of the human immunodeficiency virus protease inhibitor nelfinavir and chemoradiation for locally advanced pancreatic cancer. *J Clin Oncol.* 2008; 26(16): 2699–2706.
[PubMed Abstract](#) | [Publisher Full Text](#)
89. Rengan R, Mick R, Pryma D, *et al.*: A phase I trial of the HIV protease inhibitor nelfinavir with concurrent chemoradiotherapy for unresectable stage IIIA/IIIB

- non-small cell lung cancer: a report of toxicities and clinical response. *J Thorac Oncol*. 2012; 7(4): 709–15.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
90. Gills JJ, Dennis PA: **Perifosine: update on a novel Akt inhibitor.** *Curr Oncol Rep*. 2009; 11(2): 102–10. Review.
[PubMed Abstract](#) | [Publisher Full Text](#)
 91. Wan X, Harkavy B, Shen N, *et al.*: **Rapamycin induces feedback activation of Akt signaling through an IGF-1R-dependent mechanism.** *Oncogene*. 2007; 26(13): 1932–40.
[PubMed Abstract](#) | [Publisher Full Text](#)
 92. Sarbassov DD, Guertin DA, Ali SM, *et al.*: **Phosphorylation and regulation of Akt/PKB by the rictor–mTOR complex.** *Science*. 2005; 307(5712): 1098–1101.
[PubMed Abstract](#) | [Publisher Full Text](#)
 93. Carracedo A, Baccella J, Pandolfi PP: **Deconstructing feedback-signaling networks to improve anticancer therapy with mTORC1 inhibitors.** *Cell Cycle*. 2008; 7(24): 3805–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 94. Bernstein WB, Dennis PA: **Repositioning HIV protease inhibitors as cancer therapeutics.** *Curr Opin HIV AIDS*. 2008; 3(6): 666–75.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 95. Bernhard EJ, Brunner TB: **Progress towards the use of HIV protease inhibitors in cancer therapy.** *Cancer Biol Ther*. 2008; 7(5): 636–637.
[PubMed Abstract](#) | [Publisher Full Text](#)
 96. Cho HY, Wang W, Jhaveri N, *et al.*: **Perillyl alcohol for the treatment of temozolomide-resistant gliomas.** *Mol Cancer Ther*. 2012; 11(11): 2462–72.
[PubMed Abstract](#) | [Publisher Full Text](#)
 97. Gupta AK, Lee JH, Wilke WW, *et al.*: **Radiation response in two HPV-infected head-and-neck cancer cell lines in comparison to a non-HPV-infected cell line and relationship to signaling through AKT.** *Int J Radiat Oncol Biol Phys*. 2009; 74(3): 928–33.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 98. Gupta AK, Wilke WW, Taylor EN, *et al.*: **Signaling pathways in adenoid cystic cancers: implications for treatment.** *Cancer Biol Ther*. 2009; 8(20): 1947–51.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 99. Danaher RJ, Wang C, Roland AT, *et al.*: **HIV protease inhibitors block oral epithelial cell DNA synthesis.** *Arch Oral Biol*. 2010; 55(2): 95–100.
[PubMed Abstract](#) | [Free Full Text](#)
 100. Kimple RJ, Vaseva AV, Cox AD, *et al.*: **Radiosensitization of epidermal growth factor receptor/HER2-positive pancreatic cancer is mediated by inhibition of Akt independent of ras mutational status.** *Clin Cancer Res*. 2010; 16(3): 912–23.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 101. Bourlier V, Zakaroff-Girard A, De Barros S, *et al.*: **Protease inhibitor treatments reveal specific involvement of matrix metalloproteinase-9 in human adipocyte differentiation.** *J Pharmacol Exp Ther*. 2005; 312(3): 1272–1279.
[PubMed Abstract](#) | [Publisher Full Text](#)
 102. Thomas S, Sharma N, Golden EB, *et al.*: **Preferential killing of triple-negative breast cancer cells *in vitro* and *in vivo* when pharmacological aggravators of endoplasmic reticulum stress are combined with autophagy inhibitors.** *Cancer Lett*. 2012; 325(1): 63–71.
[PubMed Abstract](#) | [Publisher Full Text](#)
 103. Wang X, Sato R, Brown MS, *et al.*: **SREBP-1, a membrane-bound transcription factor released by sterol-regulated proteolysis.** *Cell*. 1994; 77(1): 53–62.
[PubMed Abstract](#) | [Publisher Full Text](#)
 104. Shimomura I, Hammer RE, Richardson JA, *et al.*: **Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: model for congenital generalized lipodystrophy.** *Genes Dev*. 1998; 12(20): 3182–3194.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 105. Riddle TM, Kuhel DG, Woollett LA, *et al.*: **HIV protease inhibitor induces fatty acid and sterol biosynthesis in liver and adipose tissues due to the accumulation of activated sterol regulatory element-binding proteins in the nucleus.** *J Biol Chem*. 2001; 276(40): 37514–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
 106. Guan M, Fousek K, Jiang C, *et al.*: **Nelfinavir induces liposarcoma apoptosis through inhibition of regulated intramembrane proteolysis of SREBP-1 and ATF6.** *Clin Cancer Res*. 2011; 17(7): 1796–806.
[PubMed Abstract](#) | [Publisher Full Text](#)
 107. Lenhard JM, Croom DK, Weiel JE, *et al.*: **HIV protease inhibitors stimulate hepatic triglyceride synthesis.** *Arterioscler Thromb Vasc Biol*. 2000; 20(12): 2625–2629.
[PubMed Abstract](#) | [Publisher Full Text](#)
 108. Crum-Cianflone NF, Hulsiek KH, Marconi V, *et al.*: **The impact of nelfinavir exposure on cancer development among a large cohort of HIV-infected patients.** *J Acquir Immune Defic Syndr*. 2009; 51(3): 305–9.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 109. Giardino Torchia ML, Ciaglia E, Masci AM, *et al.*: **Dendritic cells/natural killer cross-talk: a novel target for human immunodeficiency virus type-1 protease inhibitors.** *PLoS One*. 2010; 5(6): e11052.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 110. Lucia MB, Anu R, Handley M, *et al.*: **Exposure to HIV-protease inhibitors selects for increased expression of P-glycoprotein (ABCB1) in Kaposi's sarcoma cells.** *Br J Cancer*. 2011; 105(4): 513–22.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 111. Garcia MG, Alaniz LD, Cordero Russo RI, *et al.*: **PI3K/Akt inhibition modulates multidrug resistance and activates NF- κ B in murine lymphoma cell lines.** *Leuk Res*. 2009; 33(2): 288–296.
[PubMed Abstract](#) | [Publisher Full Text](#)
 112. Hui DY: **Effects of HIV protease inhibitor therapy on lipid metabolism.** *Prog Lipid Res*. 2003; 42(2): 81–92.
[PubMed Abstract](#) | [Publisher Full Text](#)
 113. You J, He Z, Chen L, *et al.*: **CH05-10, a novel indinavir analog, is a broad-spectrum antitumor agent that induces cell cycle arrest, apoptosis, endoplasmic reticulum stress and autophagy.** *Cancer Sci*. 2010; 101(12): 2644–51.
[PubMed Abstract](#) | [Publisher Full Text](#)
 114. Maksimovic-Ivanic D, Mijatovic S, Miljkovic D: **The antitumor properties of a nontoxic, nitric oxide-modified version of saquinavir are independent of Akt.** *Mol Cancer Ther*. 2009; 8(5): 1169–78.
[PubMed Abstract](#) | [Publisher Full Text](#)
 115. Yorititsu T, Nair U, Yang Z, *et al.*: **Endoplasmic reticulum stress triggers autophagy.** *J Biol Chem*. 2006; 281(40): 30299–304.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 116. Sato A, Asano T, Ito K: **Ritonavir interacts with bortezomib to enhance protein ubiquitination and histone acetylation synergistically in renal cancer cells.** *Urology*. 2012; 79(4): 966.e13–966.e21.
[PubMed Abstract](#) | [Publisher Full Text](#)
 117. McLean K, VanDeVen NA, Sorenson DR, *et al.*: **The HIV protease inhibitor saquinavir induces endoplasmic reticulum stress, autophagy, and apoptosis in ovarian cancer cells.** *Gynecol Oncol*. 2009; 112(3): 623–30.
[PubMed Abstract](#) | [Publisher Full Text](#)
 118. Kawabata S, Gills JJ, Mercado-Matos JR, *et al.*: **Synergistic effects of nelfinavir and bortezomib on proteotoxic death of NSCLC and multiple myeloma cells.** *Cell Death Dis*. 2012; 3: e353.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

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Ansgar Brüning

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This is a highly informative and comprehensive review on our current knowledge about the anti-cancer mechanism of nelfinavir. It also deals with the pros and cons of a possible repurposing of nelfinavir (and other HIV PIs) for cancer treatment.

The following amendments and corrections are suggested:

Proteasome: In the abstract, the proteasome is listed among the factors to be “down-regulated” by nelfinavir. Is the proteasome “downregulated” or “inhibited” in its activity? This might be a slight semantic inaccuracy but is important because it reflects different molecular mechanisms. The same applies to Akt, which, according to the author, is mentioned to be “inhibited” in some phrases and to be “downregulated” (as a protein?) by nelfinavir in other phrases.

Independent of this, there is also a controversy in the literature about the ability of nelfinavir to inhibit proteasomal activity. Some authors observed inhibition of proteasomal enzyme activity by HIV PIs, whereas proteasomal target genes such as HIFalpha and cyclins have unexpectedly not been described to accumulate in the presence of PIs. Instead, as mentioned in this review, several of these proteasomal target proteins were found to be downregulated by nelfinavir treatment.

Using yeast terminology (HAC1) to introduce human ER stress mechanisms might confuse the reader.

Fig.1: How does ER stress lead to proteasome inhibition?

I have problems with the author’s proposed classification of PIs into ER stress inducers and Akt inhibitors (Abstract and Conclusion). Ritonavir is listed as an ER stressor only (conclusions “9”), but has been identified by several researchers to cause both ER stress and Akt inhibition.

Furthermore, according to our experience, the ability of PIs to reduce Akt activity appears to be dependent on the cell line(s) used. Our group also observed enhanced Akt phosphorylation by nelfinavir in cell lines with low endogenous Akt activity, and Akt activation is also a known

response to ER stress.

A combination of nelfinavir with mTOR inhibitors is suggested by the author. There are some independent recent reports indicating that nelfinavir also acts as an mTOR inhibitor.

The ability of nelfinavir to induce oxidative stress should be mentioned, because this may represent one of the reasons why nelfinavir may exert ER stress or other anti-cancer effects. (Fig. 2, for example, suggests undefined “protease inhibition” as the only mechanism of nelfinavir-induced ER stress).

Some typos in the text and in the figures should be corrected (hidroxychloroquine; phosphorylation; endothelial (=endoplasmic?) reticulum; Bc12 (=bcl2? Fig. 5)).

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 03 Jun 2015

Tomas Koltai, Centro de Diagnostico y Tratamiento de la Obra Social del Personal de la Alimentación, Talar de Pacheco, Buenos Aires, Argentina

Thank you for your deep and knowledgeable revision of this manuscript. I agree with you about the inaccuracies mentioned and there will be corrected in a third version of the article.

Of course, down-regulation and inhibition in the molecular world are two different things, so it is wise to make the modifications you suggested: proteasome is inhibited and not down-regulated by NFV. Akt, being a protein is down-regulated and not inhibited. These will be modified in version 3.

Yeast terminology for proteins playing a role in proteosomal stress may be confusing, so it will be changed for proteins found in mammalian proteosomal stress.

The classification of PIs according their main anticancer activity is based on clinically achievable concentration of the drug. It is quite possible (I found no experimental evidence so far) that at very high concentrations, PIs are both ER stressors and Akt down-regulators. But at doses used in the clinical setting the classification I propose may represent actual differences between the different PIs.

Oxidative stress will be mentioned in version 3. I agree it is an important cause of ER stress.

Amendments will be made to figure 5 along with the remarks you kindly made. The association of NFV and mTOR inhibitors showed interesting results in naturally occurring squamous cell tumors in dogs in our experience (unpublished data). This probably means some sort of synergy between the two. This synergy is mentioned by many authors. NFV acting on mTOR will be particularly highlighted.

Thank you for your interesting revision. All issues you mentioned will be considered in version 3 of this article.

Competing Interests: No competing interests

Version 1

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The article by Koltai reviews the extensive literature concerning Nelfinavir as an anti-cancer drug. Overall the study makes a good update of the evidences of nefilnavir anti-cancer activity and its role in controlling ERS-UPR pathway. It would be interesting to include in the discussion information about the different trials that are currently carry on with Nelfinavir in different tumors.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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