Antitumorigenic action of nelfinavir: Effects on multiple myeloma and hematologic malignancies (Review)

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Abstract. Protease inhibitors (PIs) inhibit HIV-1 and HIV-2 proteases, impeding virus replication and liberation of viral elements from infected cells. In human immunodeficiency virus (HIV) subjects receiving PI-based treatment, an impressive decrease in the amount of HIV-associated cancers, unconnected to viral burden or CD4 amount was observed. Research has reported that PIs have influence on cancer proliferation, spread, and survival as an effect on endoplasmic reticulum stress, proteasome, NF-κB and Akt signalling. Nelfinavir (NFV) is a nonpeptidic PI that functions by connecting to the catalytic site of the HIV protease, thus stopping the cleavage of viral polyprotein into complete, operative proteins that are fundamental for viral survival. NFV, currently not frequently employed for antiretroviral treatment, has demonstrated noteworthy off target effects in tumor patients with or without HIV disease. NFV appears to cause cell death in tumor cells by different mechanisms, which include necrosis, apoptosis and autophagy. In this review, data from preclinical research and clinical trials are reported and the mechanisms of action of NFV and their results in the treatment of hematologic malignancies, such as acute myeloid leukemia, chronic lymphoid leukemia, and diffuse large B cell lymphoma, and especially in patients with multiple myeloma are examined. In the future, experimental studies may help identify the role of NFV in cancer treatment and may promote the application of this drug into daily clinical practice.

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Contents

1. Introduction: General considerations on protease inhibitors

Protease inhibitors (PIs) inhibit human immunodeficiency virus (HIV)-1 and HIV-2 proteases, impeding viral replication and liberation of viral elements from infected cells. The mechanism of action of PIs involves competitive binding to the enzyme. PIs were designed to halt the development of the HIV virion, by impeding cleavage of polyproteins by the viral aspartyl protease into their operational layout. Since 1995, by employing the HIV protease crystal layout, various small inhibitors have been constructed for HIV therapy. To date, numerous PIs have been produced including ritonavir, amprenavir, lopinavir, ritonavir, atazanavir, indinavir, darunavir, fosamprenavir and tipranavir. Their use has caused a decrease in the mortality rate due to HIV infection, and a reduction in the prevalence of opportunistic infections (1). In fact, with the use of PIs and of HIV reverse transcriptase inhibitors the era of highly active antiretroviral treatment (HAART) started, which is now the main therapy in HIV infection. Moreover, in HIV subjects receiving PI-based treatment, an impressive decrease in the amount of HIV-associated cancers, unconnected to the viral burden or CD4 amount, has been observed. This has paved the way for a series of studies aimed at evaluating the effects of PIs on neoplastic diseases. It has been therefore demonstrated that numerous PIs have consequences on cancer proliferation, spread and outcome. This is possibly due to actions on endoplasmic reticulum (ER) stress, the proteasome, NF-κB and Akt signaling (2,3).

Employing drugs used for the treatment of infectious diseases to cure tumors has been noted in several previous cases, such as fludarabine and tetracycline, which have demonstrated action

on women with breast cancer (4). The possible use of anti-HIV drugs against cancer is also not new. Research has demonstrated that PIs display anticancer action which is independent from their capacity to interfere with the HIV protease. Ikeoze *et al* demonstrated that ritonavir, indinavir and saquinavir mediated a decrease in the growth and differentiation of HL-60 and NB4 myeloid leukemia cells (5). Other studies have shown that PIs can reduce the growth of Kaposi sarcoma, urological cancer cells and pheocromocytoma cells (5-8).

Notwithstanding their partial similarity with the HIV protease, cellular proteases appear to be the main target of PIs that are responsible for their anticancer action. PIs particularly act on the proteasome and extracellular matrix metalloproteases (1). Nevertheless, several of the supplementary actions of PIs have been demonstrated. PIs can reduce angiogenesis via a reduction in the phosphoinositide 3-kinase (PI3K)/Akt pathway, which regulates the production of vascular endothelial growth factor (VEGF) (9,10), increase apoptosis in cancer cells by inhibition of STAT3 and c-Src. Moreover, PIs may block NF-κB activation via a reduction in proteasomal degradation of IαB (6,11-14). However, the actions appear to be cell type-dependent; for instance, ritonavir may be protective against cell death in normal T cells (15).

A fundamental work by Gills *et al* demonstrated that PIs displayed an effect against all 60 cell lines in the NCI60 panel and the authors reported several possible mechanisms of action of PIs against tumor cells (16).

2. Nelfinavir

In March 1997, the Food and Drug Administration approved nelfinavir (NFV) for HIV treatment. NFV is an orally available drug used against HIV-1 and HIV-22 (17) (Fig. 1). NFV is a nonpeptidic PI that works by binding to the catalytic site of the HIV protease, thus impeding the maturation of viral polyprotein precursors into operational proteins that are indispensable for viral proliferation. NFV is present in tablets of 250 and 625 mg or as an oral suspension powder. The suggested dose of NFV for adult patients is 1,250 mg twice or 750 mg three times a day (17).

Currently, NFV is infrequently employed for antiretroviral therapy, as it is being replaced by second-generation HIV PIs but has demonstrated beneficial effects in tumor patients with or without HIV infection (Fig. 2).

A previous study demonstrated that PIs have action against cancer cells, with NFV being the most powerful among the drugs tested (16). A complete computational study of protein interactions has discovered 92 predicted cellular targets of NFV, among which there were 7 with the highest binding affinities and they were aspartyl proteases (18). The residual targets were growth factor receptors that regulate NF-κB, Akt and other signaling molecules (19).

Indeed, NFV appears to be the most effective inhibitor of Akt, even though this differs by cellular type (20). In rapamycin-resistant diffuse large B cell lymphoma cell lines in which Akt was upregulated, the use of NFV with Akt inhibitor MK-2206 resulted in increased cytotoxicity (21,22).

A different mechanism through which NFV could express its antineoplastic action could be its action on angiogenesis. NFV can block angiogenesis via the downregulation of PI3K/Akt, which regulates the expression of VEGF and other elements implicated in cancer neovascularization (10). However, the concentration of Akt does not constantly correlate with the anticancer action, and in some experimental models NFV paradoxically stimulated Akt (23). This may result from a reduction in growth factors or an increase in endoplasmic reticulum (ER) stress. In fact, the most relevant anticancer action of NFV is due to ER stress and unfolded protein response (UPR) which may be one of the means resulting in cell death (24). NFV blocks the proteases S1P and S2P that are implicated in SREBP-1 maturation and other proteases essential for protein folding in ER (25).

Shim *et al* and Srirangam *et al* studied different breast cancer cell lines and demonstrated that NFV reduced the growth of human epidermal growth factor receptor 2 (HER2)-positive breast cancer cells when compared to HER2-negative cells. In HER2-positive breast cancer cells, NFV provoked a degradation of HER2 and Akt by blocking their connection with heath shock protein 90 (HSP90) (26,27).

3. Nelfinavir and cancer

As stated above, NFV promotes cell death in tumor cells by different mechanisms, including necrosis, apoptosis and autophagy (16,28). The accumulation of misfolded proteins could be the main mechanism of action of NFV in glioma, ovarian cancer cells, and lung cancer (28-30).

In breast cancer cells, tamoxifen increases the antitumor action of NFV. This synergic effect was unconnected to the estrogen receptor status so that the combination of NFV and tamoxifen may be useful even in subjects with no hormone responsive tumors (31) (Table I).

In a different study, the proteasome inhibitor bortezomib and NFV were used in experiments with human cervical cancer cells. Both substances provoked cell cycle arrest in tumor cells. An increase in the molecular chaperone BiP and in cell stress marker ATF3 suggested the induction of UPR as the main mechanism of cell death in tumor cells. NFV showed no actions on proteasomal activity in the tumor cells. Even when NFV and bortezomib were active on cisplatin-resistant cervical cancer cells, neither of the two substances provoked a sensitization to cisplatin therapy. Instead, both drugs augmented the effectiveness of an apoptosis-inducing TRAIL receptor antibody (32).

In head and neck tumors, NFV promoted a reduction in Akt and radiosensitization (33), while in adenoid cystic cancer, NFV reduced Akt and MAPK (34), and decreased oral cell growth, including normal keratinocytes and squamous cancer cells (35).

Finally, there is evidence that NFV is able to act on pancreatic tumors (36), while data have demonstrated the ability of NFV to sensitize tumor cells to chemoradiotherapy (36).

4. Nelfinavir and haematological malignancies

Several studies have demonstrated the possibility of NFV to take action not only on solid neoplasms but also on haematological malignancies (Table II) such as acute myeloid leukemia (AML).

Brüning *et al* assessed the action of NFV on leukemia cells and non-malignant bone marrow-derived cells. At a dosage of 9 μg/ml, NFV caused 90% cell death of IM9, HL60, and Jurkat cells. At similar levels, less than 10% of non-malignant bone

marrow-derived cells displayed NFV-provoked cell damage. NFV-provoked death of leukemia cells was preceded by an increase in caspases 3, 7 and 8. However, despite caspase activation, the increase in the antiapoptotic bcl-2 family member protein mcl-1 that followed the NFV treatment stabilized the mitochondrial membrane potential, causing mitochondrial-independent cell death. Reduction in mcl-1 expression using sorafenib increased NFV-induced apoptosis even at minor NFV levels but did not have supplementary negative actions on non-malignant bone marrow cells (37).

Similarly, NFV exhibited a cytotoxic effect against primary AML cells, stimulated PS-caused apoptosis, blocked AKT-phosphorylation and demonstrated synergistic cytotoxicity with carfilzomib and bortezomib at micromolar levels (38). NFV blocked intracellular proteasome activity, including β2 proteasome activity that was not affected by bortezomib and carfilzomib (39).

The presence of NFV-caused cytotoxicity was also reported in pediatric leukemia cells. NFV was tested against pediatric leukemia cells by *in vitro* proliferation inhibition essays. Several substances were recognized to have a synergistic effect with NFV in its antileukemic activity such as AT101 (Bcl-2 family inhibitor), sunitinib (TK inhibitor), and JQ1 (BET inhibitor) (40).

NFV has also been shown to have a possible therapeutic action in lymphoproliferative disorders. NFV provokes moderate ER stress and autophagy in chronic lymphoid leukemia (CLL) cells. Remarkably, NFV did not cause direct cytotoxicity against CLL cells as a single drug. Nevertheless, co-treatment with NFV and chloroquine markedly provoked the direct death of CLL cells *in vitro* (41).

Diffuse large B cell lymphoma (DLBCL) is the most frequent type of Non-Hodgkin's lymphoma (NHL) (42). The mTOR pathway is constitutively stimulated in DLBCL, and blockage of mTOR is a possible treatment for DLBCL (43), although the response to mTOR inhibitors (mTORi) is approximately 30% in DLBCL (44) due to the onset of resistance to mTORi (45).

Petrich *et al* assessed DLBCL cell lines with differential resistance to the mTORi. Then the authors assessed NFV and MK-2006, chosen for their potential to synergize with rapamycin in DLBCL. Both substances demonstrated synergistic inhibition of cell viability in combination with rapamycin in DLBCL cell lines, and strongly inhibited targets of activated mTOR (21).

Another research study investigated the possibility that IPs may modify the kinetics of drugs employed in lymphoma treatment. Comprehensive pharmacokinetics and pharmacodynamic analysis were carried out in 19 NHL subjects during 38 cycles of chemotherapy: 19 cycles with CHOP and 19 CHOP + HAART. Highly active antiretroviral therapy (HAART) comprised NFV, saquinavir (SQV), and indinavir (IDV). No substantial actions of HAART on the pharmacokinetic values of doxorubicin (DOX) were described. Similarly, no differential action on DOX pharmacokinetics among NFV, SQV, and IDV was demonstrated (46).

5. Nelfinavir and multiple myeloma

The hematological pathology in which the effects of NFV have been most studied is represented by multiple myeloma (MM).

Table I. Studies demonstrating the effects of nelfinavir on cancer.

Cancer cells	Actions	(Refs.)
Breast cancer cells	Synergic action with tamoxifen	31
Cervical cancer cells	Cell cycle arrest	32
Head and neck tumor	Radiosensitization	33
Adenoid cystic cancer	Reduced cell growth	34

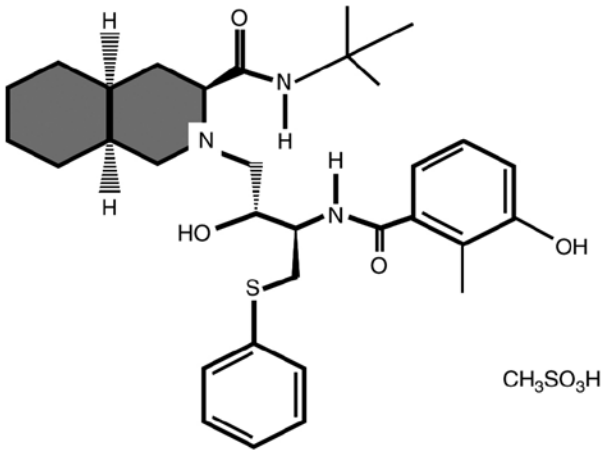

Figure 1. The chemical structure of nelfinavir.

Ikezoe *et al* demonstrated that NFV promoted suppression of proliferation and cell death of MM RPMI8226, U266, and ARH77 cell lines. This event occurred in association with a decrease in the antiapoptotic protein Mcl-1. Moreover, NFV suppressed the survival of isolated MM cells from subjects. However, NFV did not influence survival of normal bone marrow (BM)-derived cells and colony formation of myeloid committed stem cells (CFU-GM) from control subjects. Furthermore, the authors observed that NFV reduced interleukin-6 (IL-6)-stimulated phosphorylation of both signal transducer and activator of transcription 3 (STAT3) in MM cells and decreased basal and IL-6-stimulated STAT3/DNA binding activity (47).

A relevant aspect of NFV action is represented by the ability of PIs to act synergistically with the drugs used in the treatment of MM. It was demonstrated that NFV and bortezomib (BZ) synergistically increased proteotoxicity, reduced cell growth and provoked cell death in MM cells. The combination of the two drugs increased activating transcription factor (ATF)3 and CCAAT-enhancer binding protein homologous protein (CHOP), markers of ER stress, while their siRNA-mediated knockdown reduced cell death. Pre-treatment with cycloheximide (a protein synthesis inhibitor), reduced the concentrations of ubiquitinated proteins, CHOP and ATF3, indicating that reduction in protein synthesis augments cell survival by reducing proteotoxic stress. The use of NFV/BZ was found to reduce the proliferation of non-small cell lung carcinoma (NSCLC) xenografts, which was associated with the increase in markers of ER stress and cell death (48).

Table II. Studies demonstrating the effects of nelfinavir on hematologic malignancies.

Tumor cell lines and patients	Effect	(Refs.)
Leukemia cells	Increased cell death	37
Primary acute myeloid leukemia	Increased apoptosis	38
Pediatric leukemia cells	Proliferation inhibition	40
Chronic lymphoid leukemia	Increase of autophagy	41
Diffuse large B cell lymphoma cell lines	Inhibition of cell viability	21
MM cell lines	Increased cell death	47
Phase I trial	Overcomes proteasome inhibition resistance in MM patients	60
Phase I/II trial in MM patients	Overcomes lenalidomide resistance in MM patients	66
Phase II trial in MM patients	ORR 65%	61

MM, multiple myeloma; ORR, objective response rate.

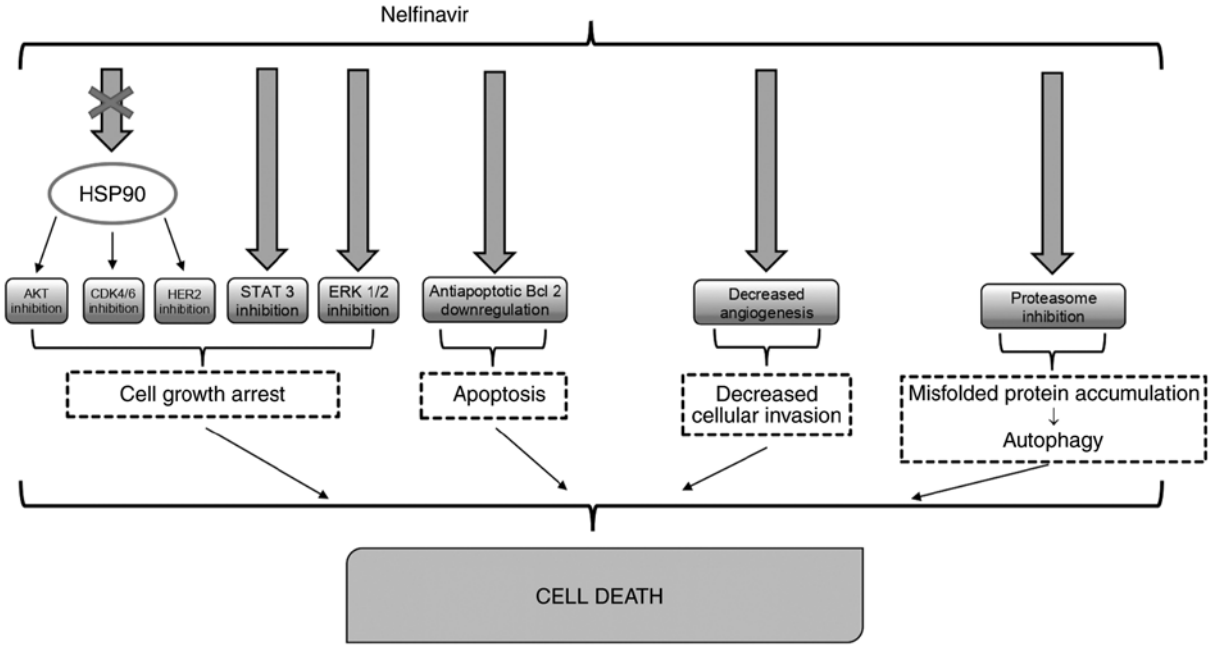

Figure 2. Possible actions of nelfinavir in neoplastic diseases. AKT, protein kinase B; CDK4/6, cell division protein kinase 4/6; HER2, human epidermal growth factor receptor 2; STAT3, signal transducer and activator of transcription 3; Bcl2, B-cell lymphoma 2.

Proteasome inhibitors are the mainstay of MM treatment. Nevertheless, certain MM patients acquire proteasome inhibitor resistance. It is well known that the proteasome inhibitor sensitivity of MM cells is controlled by UPR (49,50), that avoids accumulation of altered proteins in the ER by operating on mRNA translation and protein destruction. This is controlled by ER-associated degradation machinery, with the proteasome as its rate-limiting terminal protease (51). Extreme activation of UPR causes apoptosis and this is the main mechanism of action of BZ in MM patients (52). The concentration of UPR pre-activation regulates the proteasome inhibitor-sensitivity of MM, so that an increase in UPR may overcome proteasome inhibitor resistance (53). Activation of UPR is started via inositol-requiring kinase 1 (IRE1) (54). Full plasma cell maturation requires UPR activation via the IRE1/XBP1 axis and causes a mature, proteasome inhibitor-sensitive MM cell type. On the contrary, IRE1-/XBP1-MM cells are proteasome inhibitor-resistant and are increased in proteasome inhibitor-resistant MM subjects. However, while IRE1-targeting drugs are at an early stage of assessment (55), it has been demonstrated that NFV has UPR- and IRE1/XBP1-stimulating activity (24,56). This activity may interest interference with UPR-stimulating proteases (57), the AKT pathway (58) and the proteasome (59). It is able to re-sensitize proteasome inhibitor-resistant MM cells at low micromolar levels (38).

In addition to preclinical studies, numerous clinical trials have attempted to demonstrate the efficacy of NFV in patients with MM. In a phase I trial (SAKK 65/08), the authors demonstrated that NFV blocked proteasome activity and increased the amount of proteins correlated to UPR in peripheral blood mononuclear cells. Contemporary use of NFV with BZ further stimulated UPR and overwhelmed proteasome inhibitor resistance (60).

In another study, the authors performed a phase 2 trial (SAKK 39/13) to study the effects and safety of NFV in MM refractory subjects to proteasome inhibitors and previously treated with immunomodulatory drugs. The protocol provided for administration of NFV 2,500 mg on days 1-14 twice daily; bortezomib 1.3 mg/m^2 i.v./subcutaneously on days 1, 4, 8 and 11; and dexamethasone 20 mg orally on days 1-2, 4-5, 8-9 and 11-12 for up to six 21-day cycles. The results of the study were remarkable. Patients were heavily pre-treated, but ORR was 65%, a rate comparable to the one reported in first-line BZ-naïve subjects. Moreover, it is possible that clinical advantage was underestimated as the treatment was administered only for 4.2 months (61).

NFV has proteasome-blocking action at high levels (20-40 μM) *in vitro* (62), but peak NFV levels at the 2x1,875 mg dose are in the 15 μM range in treated subjects (60). Besse *et al* conjectured that adjoining lenalidomide to NFV therapy may augment intracellular NFV levels necessarily to cause the pan-proteasome-blocking action seen with high levels of NFV. Both NFV and lenalidomide are substrates of MDR-1 type drug efflux pumps (63); thus, competition of the two substances for the MDR-1 drug pump may reduce the efflux of NFV. Similarly, NFV may augment the efficacy of the treatment by increasing intracellular lenalidomide levels within myeloma cells (63). Similar considerations can also be made for the simultaneous administration of NFV with carfilzomib, a different proteasome inhibitor (64,65).

A phase I/II trial evaluated whether adjoining NFV to lenalidomide-dexamethasone can overcome lenalidomide resistance in MM patients. Twenty-nine subjects were studied (lenalidomide-BZ double-refractory 34%). They were treated with four cycles of NFV 2,500 mg/day with lenalidomide 25 mg days 1-21 and dexamethasone (40/20 mg days 1, 8, 15 and 22). It was shown that a minor response was attained in 55% of patients, while a partial response was achieved in 9 patients (31%). Median overall survival was 21.6 months. Peripheral blood mononuclear cells exhibited a 45% (95% CI, 40-51%) decrease in total proteasome activity and substantial increase of UPR and autophagy. Thus, NFV/lenalidomide/dexamethasone appears to be an active oral treatment for lenalidomide-refractory MM (66).

6. Nelfinavir and its disadvantages

Severe adverse effects of PIs are infrequent with the exclusion of diarrhea when employed at high dosages. Nevertheless, there are other unfavorable side events with PIs, such as insulin resistance, hyperlipidemia and lypodystrophy. The main factor underlying these side events is the suppression of the breakdown of sterol regulatory element binding proteins (SREBP) in the liver and adipose tissues causing an increased cholesterol and fatty acid biosynthesis. SREBP storage in adipose tissue provokes lipodystrophy. Moreover, PIs reduce proteasome-mediated breakdown of lipoprotein (apo) B, causing an increase in the production of triglyceride. Finally, NFV also reduces storage of the glucose transporter GLUT-4 in adipose tissue. This may promote the onset of insulin resistance and diabetes (67).

Moreover, an increase in serum aminotransferase may occur in subjects receiving NFV. Considerable increase in serum aminotransferase levels (>5 times) is present in 3-10% of subjects. This increase is generally asymptomatic and self-limited and does not require suspension of the drug. Hepatomegaly and hepatic steatosis are direct effects of the metabolic changes reported above (68). Clinical features of hypersensitivity such as rash, fever, of eosinophilia can arise as autoantibody formation but these events are not very noticeable. Finally, it was reported that myelosuppression is more common in subjects treated with chemotherapy and HAART combination (69).

However, despite the substantially good tolerability of treatment with NFV, some issues must be solved. Although NFV has pro-apoptotic activity on tumor cells, an increase was reported in the antiapoptotic mitochondrial membrane protein mcl-1 able to increase phosphorylation of ERK1/2 (extracellular signal-regulated kinases 1/2) (70). Upregulation of ERK is able to reduce cell death. This condition can be solved with the administration of sorafenib (37,70).

Moreover, in spite of the antitumor action of NFV, this drug does not decrease the risk of tumor onset in HIV patients and also causes a reduction in immunological functions, altering the differentiation of monocytes into dendritic cells (71,72). Finally, although the results from numerous studies demonstrate that the combination of HAART with chemotherapy improves prognosis, many uncertainties exist on the choice of the best combination of chemotherapy and PIs. Overlapping toxicities have been reported with combination treatment, and there is a possible risk for pharmacokinetic associations between chemotherapy and PIs (73).

7. Future perspectives

Although the use of NFV has given encouraging results in *in vitro* and *in vivo* studies, even better results could be achieved by using drug combinations. Akt activation plays a main role in the tumor phenotype (74). At present there are no substances able to inhibit this protein with a convenient safety profile. The anti-Akt action of NFV can be increased by simultaneous mTOR blockage which provokes a synergistic cytotoxicity (21). This may be since mTOR inhibition without Akt blockage removes a negative biofeedback loop on Akt, causing increased phosphorylation of Akt (75,76). The negative bio-feedback loop on Akt must be solved to achieve appropriate results. NFV could be useful to obtain better results when used with mTORi.

Numerous other substances have been identified as possible synergists with NFV. NFV increases anti-malarial activity of artemisinin *in vitro* on *Plasmodium falciparum*, but artemisinin also has antitumor action (77). Several studies have tried to evaluate whether the simultaneous administration of artemisinin and NFV could enhance the antineoplastic action of the two substances. A research study employed NFV and artemisinin, in an experimental protocol (CUSP 9) for the therapy of relapsed glioblastoma. The combination was reported to postpone glioblastoma spread (78).

Experimental tests are however necessary to verify the possibility that the use of NFV with other drugs such as celecoxib or chloroquine, may be effective for the treatment of neoplasms. In fact, the cancer cell killing capacity of NFV can be increased with different ER stressors such as celecoxib (79); perillyl alcohol is another stress factor that has been employed using this rationale (80).

Hydroxicloroquine and chloroquine are autophagy inhibitors and may also operate synergistically with NFV, decreasing autophagy and augmenting apoptosis (41,81,82).

8. Conclusions

Drug repositioning leads to the identification of new indications for current drugs and the use of the newly recognized medicines to therapy of diseases other than the drug's intended target. Even though the exact molecular target of NFV is still to be identified, its efficacy and safety are well known. All data reported in this review support the hypothesis that NFV is a useful means of integration in cancer treatment. In the future, it should be evaluated in combination with chemotherapy in the design of new protocols. Indeed, it is fundamental to establish the most appropriate associations, dosing and timing of NFV administration in patients with MM or other hematological diseases. In fact, concurrent therapy with PIs is not without drug interactions. As such, clinical decisions regarding therapy should be carefully evaluated, and dose adjustments must be made to reduce the risk for adverse outcomes and disease progression. Moreover, new PIs are being created with better anticancer activity and further development of new PIs with stronger anticancer activity will be realized in the future.

Despite the above, research for the use of antivirals in the treatment of hematologic neoplasias and in particular MM is ongoing. In fact, notwithstanding recent advanced therapy opportunities such as proteasome inhibitors, histone deacetylase inhibitors, immunomodulatory drugs and immunotherapy, and myeloma-targeted antibodies (83-87), MM is still judged as an incurable disease.

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Authors' contributions

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Ethics approval and consent to participate

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Patient consent for publication

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Competing interest

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