# Role of antidiarrheal agents nifuroxazide in antitumor multi-target anticancer, multi-mechanism anticancer drug (Review)

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**Abstract.** Nifuroxazide (NFZ) is an antimicrobial drug, which has been found to be a promising antitumor agent in recent years. In addition to being a classic STAT3 inhibitor, NFZ can also act on IL-6 and exert an anti-tumor role through inflammatory factor pathways. It can also bind to target proteins of aldehyde dehydrogenase 1, one of the families of E-twenty-six transcription factors and ubiquitin-specific protease 21 to play an anti-tumor role in different pathways. NFZ is able to act on the tumor cell microenvironment to inhibit tumor angiogenesis and tumor cell migration, enhance tumor immune cells, increase the cytotoxicity of tumor cells and enhance the anti-tumor effect of other drugs. Furthermore, it has high safety with few toxic side effects. The anti-tumor mechanisms of NFZ were described in the current review, aiming to provide insight and a reference for future studies promoting the implementation of NFZ as an anti-tumor drug in the clinic.

## Contents



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*Abbreviations:* NFZ, nifuroxazide; JAK2, Janus kinase 2; STAT3, signal transducer and activator of transcription 3; IL-6, interleukin-6; ALDH1, aldehyde dehydrogenase 1 family, member A1; ERG, v-ets avian erythroblastosis virus E26 oncogene related; UPS-21, ubiquitin-specific protease 21

*Key words:* nifuroxazide, STAT3, tumor, IL-6, ALDH, ERG, tumor microenvironment

## 1. Introduction

Nifuroxazide (NFZ) is a gastrointestinal antibiotic that was first patented by Laboratoires Robert & Carriere SA (France) in 1961 (France) and 1966 (USA) (1). It was widely used and heavily promoted in the 1970s. When taken orally, the drug can be absorbed and metabolized in the liver (2). NFZ was first defined as a broad-spectrum intestinal antibiotic for the effective and safe treatment of bacterial vaginitis, chlamydia trachomatis, mycoplasma and candida infections (3). NFZ has a long history of clinical application. In 2008, NFZ was identified as a potent inhibitor of signal transduction and transcriptional activation factor 3 (STAT3)-dependent gene expression by screening 1,200 bioactive compounds in the Prestwick library (4). NFZ is defined as a STAT3 inhibitor. A study has shown that this kinase inhibitory activity explains the anti-proliferative activity of NFZ in myeloma cells, which performs a constitutive activation of STAT3 with negligible effects on normal cells (4). Recent studies have shown that NFZ has obvious anti-tumor effects and numerous studies have been conducted on its anticancer activity (5-7).

The present review used PubMed (https://PubMed.NCBI.NLM.nih.gov/) to retrieve data. Key words searched included 'nifuroxazide', 'tumor', 'STAT3', 'tumor microenvironment', 'inflammatory factors' and 'anti-tumor'. The time frame for these basic studies is ~10 years, with only few studies extending to 10-20 years. This article reviews the specific mechanism of NFZ various aspects (cell cycle, target protein, drug combination, safety, etc).

## 2. NFZ blocks tumor cell cycle, inhibits tumor cell proliferation and induces tumor cell death

NFZ can downregulate cyclin D, resulting in the failure of cells to enter the S phase from G1-G0 phase, and the cell cycle stagnates at G1-G0 in a concentration-dependent manner. It has anti-melanoma activity *in vitro* and *in vivo*, has strong anti-proliferation activity against a variety of tumor cells, can induce G2/M phase arrest and cell apoptosis, and inhibit the migration and invasion of tumor cells (8). It can also significantly inhibit tumor growth, reduce cell proliferation and metastasis, and induce cell apoptosis in tumor-bearing mouse models (9).

## 3. NFZ exerts anti-tumor effects by targeting IL-6, STAT3, aldehyde dehydrogenase (ALDH), one of the families of E-twenty-six transcription factors (ERG) and ubiquitin-specific protease (USP)-21

NFZ is a multi-target drug that can fight tumors through multiple target proteins and target pathways.

*NFZ exerts antitumor effects through the JAK/STAT3 pathway.* The JAK-STAT pathway has a variety of roles in physiological processes such as cell growth, differentiation and immune response. It plays a role in cell cycle regulation, cytokine signaling and apoptosis (10). STAT3 is present in numerous malignant tumor cells. STAT3 is continuously activated in most human primary cancer sites and tumor cell lines, such as human astrocytoma, multiple osteosarcoma, prostate cancer, colon cancer, stomach cancer, liver cancer and breast cancer (5,11-17). Dysregulation of this pathway is closely related to carcinogenesis and poor prognosis of various cancers, including kidney cancer (18), lung cancer (19), cervical cancer (20) and bladder cancer (21). Immunohistochemical results of 149 patients with invasive bladder cancer showed that 51.3% of them had high STAT3 expression (21).

The amide group of NFZ is located in the para-position of the hydroxyphenyl part. This configuration promotes conjugation between carboxyl oxygen atoms, phenyl radicals and hydroxyl oxygen atoms. This structural arrangement makes the hydrogen atoms in the hydroxyl part more fluid and is a classical STAT3 inhibitor that can inhibit JAK/STAT3 phosphorylation (3,5,9,12,22-25). NFZ has antitumor effects in hematologic tumors and solid tumors by inhibiting the STAT3 signaling pathway (4,11). NFZ inhibits the constitutive phosphorylation of STAT3 by reducing the self-phosphorylation of JAK and leads to the downregulation of the target gene of STAT3, myeloid cell leukemia-*1,* thereby reducing the survival activity of myeloma cells without affecting normal peripheral blood mononuclear cells (4).

AutoDock Vina (version 1.1.2) was used for molecular docking on STAT3. The mesh sizes of catalytic and allosteric sites were 15, 15 and 22.5, respectively. The dimensions x, y and z were 11.25, 15 and 15, respectively. The Lamarque genetic algorithm was used to perform 2,500 evaluations and 100 run docking simulations. Bio via Discovery Studio Visualizer 2020 was used to show molecular interactions between proteins and ligands. Molecular docking techniques were used to dock the spatial structure of STAT3 (Fig. 1). When NFZ and Stat3 bind (binding sites Lys591, Glu612 and SER613), it can inhibit STAT3 phosphorylation and thus inhibit transcriptional pathways related to this pathway. Binding site residues, such as Lys591, are consistent with previous structural studies of STAT3 (11).

*Anti-tumor effects through the IL-6/JAK/STAT3 pathway.* In numerous cancers, inflammatory cytokines [e.g. transcription factors (TFs)] are the most direct and promising targets (11). IL-6, discovered in 1976 (26), is a glycosylated protein composed of 184 amino acids (27) and a pleiotropic cytokine, which not only participates in immune response, but also in the basic processes such as inflammation, hematopoietic function, bone metabolism and embryonic development. Physiological processes that play important roles in a variety of diseases include cell proliferation, immune surveillance, acute inflammation, metabolism and bone remodeling (26-31). It was originally described as stimulating B lymphocytes and hepatocytes (31). Numerous human malignancies are caused by a variety of cell types, including tumor cells and stromal cells (10,25,32,33).

IL-6 binds to the IL-6 receptor and then binds to the glycoprotein 130 receptor to produce a signal transduction hexamer receptor complex. Recruited and activated JAKs phosphorylate STAT3 in turn, leading to gene regulation. Abnormal expression of IL-6 occurs in various cancer types and is associated with poor clinical prognosis and metastasis. In pathophysiological states, IL-6 mediates inflammation and regulates the MAPK, PI3K and JAK/STAT carcinogenic pathways (34). STAT3 is the main downstream regulatory signal of IL-6 in regulating inflammation and tumor transformation (28,35,36). NFZ effectively inhibited STAT3 and cancer-related inflammatory phenotypes NFZ effectively inhibits STAT3 and cancer-related inflammatory phenotypes (overexpression of Bcl-2, transcriptional activation of IL-6, and association of TNF-α, Bcl-2, EGFR, STAT3, transcription factor p65 (RELA p65), and wingless-type MMTV integration site family, member 5A (WNT5a) with cell survival); NFZ alone or in combination is used to prevent or treat tumors (37). Overexpression of IL-6, IL-6Ra and gp130, the upstream effectors of the IL-6/JAK/STAT3 pathway, may lead to abnormal activation of STAT3 (38), and IL-6 is an upstream signaling component of STAT3 (13,39,40). Secretion of IL-6 induced the expression of STAT3 target genes, such as Cyclin D1, Bcl-2, Bcl-xL, VEGF, VEGFR2 and matrix metalloproteinases (MMPs) (41-44). IL-6 and p-Stat3 were overexpressed in 83 breast cancer samples. The IL-6/JAK/STAT3 signaling pathway can promote tumor cell proliferation, angiogenesis, epithelial-to-mesenchymal transition and cancer stem cell subpopulation growth, while inhibiting the anti-tumor immune response (45-47).

In breast cancer, the IL-6 pathway is frequently activated, simultaneously promoting breast cancer metastasis and suppressing the anti-tumor immune response (47). NFZ decreased the viability of three breast cancer cell lines, MCF-7, MDA-MB-231 and 4T1 cells, and induced cancer cell apoptosis in a dose-dependent manner. Activated cleavage of caspase-3 and Bax downregulated Bcl-2. It also significantly blocked the migration of cancer cells and phosphorylated STAT3 Tyr705 to reduce the expression of MMP-2 and MMP-9. It inhibited tumor growth and blocked lung metastasis formation in mice

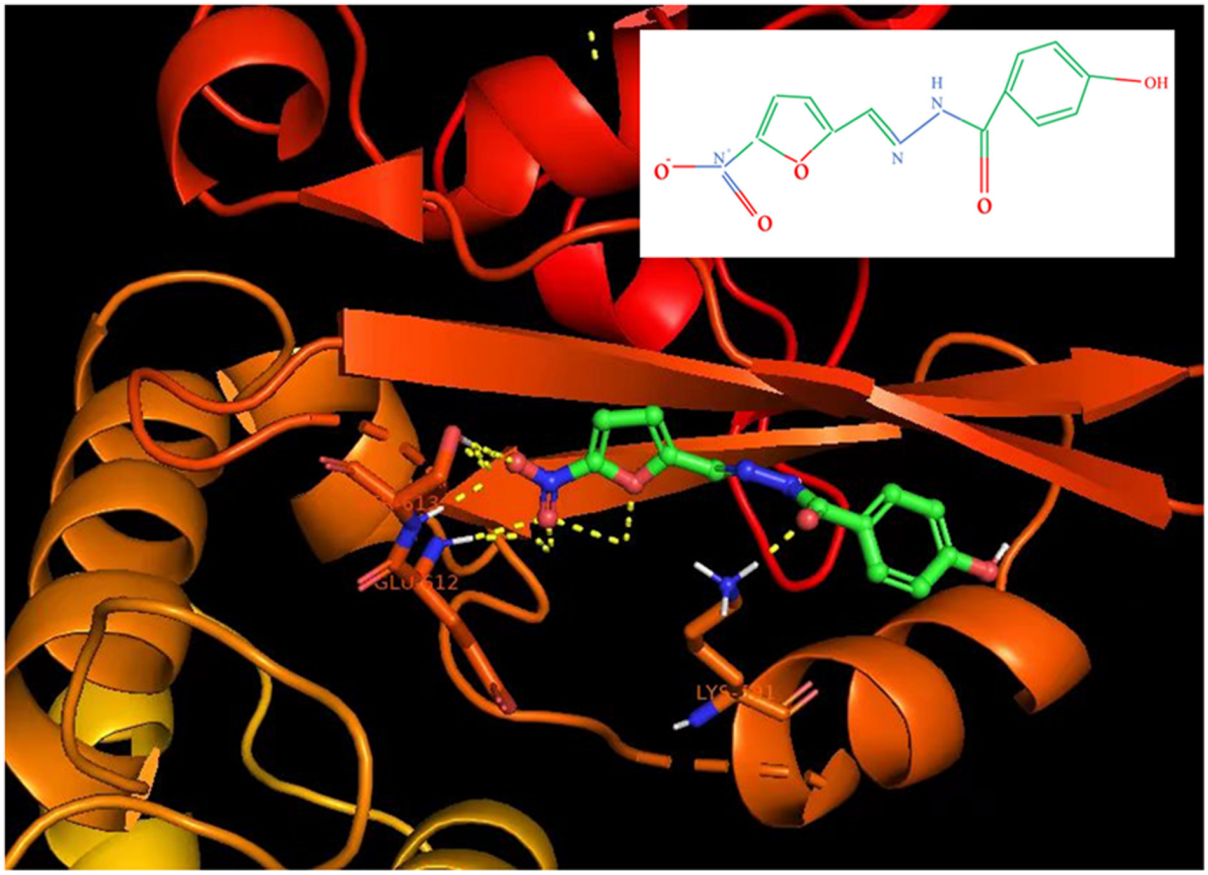


Figure 1. Molecular docking analyses of nifuroxazide and STAT3. Chemical structure of STAT3, 3D structures and interactive docking sites of STAT3 (binding sites Lys591, Glu612 and SER613).

with no detectable toxicity. The number of Ki-67-positive cells and MMP-9-positive cells can be downregulated, cleaved caspase-3 positive cells can be upregulated and the number of myelo-derived suppressor cells in the lung can be reduced by NFZ (48-52).

Abnormal STAT3 has a positive effect on breast cancer and induces G1 cell cycle progression, proliferation, anti-apoptosis, angiogenesis and metastasis through transcriptional regulation of target gene expression (39,53-57). Specific expression of STAT3 activated immunosuppressive tumor-infiltrating medullary suppressor cells (MDSCs), tumor-associated macrophages (TAM) and T-regulatory cells. STAT3 further induces upstream expression of cytokines and growth factors to produce a malignant autocrine paracrine positive feedback loop (47,57-59). STAT3 and IL-6 can further activate nuclear factor κB (NF-κB) signaling through nuclear factors in breast cancer. IL-6 is targeted to the expression of let-7 miRNAIL-6 mRNA in the 3'-untranslated region. Activation of NF-κB inhibits let-7 and leads to IL-6 hyperactivation and subsequent activation of STAT3 (60). Studies have shown that oncostatin M (OSM) can further activate the IL-6/JAK/STAT3 signaling pathway and promote the progression of breast cancer both *in vivo* and *in vitro*. OSM and IL-1β synergistically induce further activation of STAT3 secreted by IL-6 in estrogen receptor + and triple-negative breast cancer cells (54,61-63).

*IL-6/JAK/STAT3 can inhibit tumor angiogenesis.* Abnormal activation of STAT3 signaling is related to overexpression of VEGF (64,65). IL-6/STAT3 is thought to regulate the development of breast cancer by promoting JAK and angiogenic signals (66-68). NFZ can inhibit the growth of Ehrlich's breast cancer *in vivo* (11,69). Female albino mice were injected with cells from Ehrlich cancer producing Ehrlich solid tumors. NFZ was able to reduce the tumor mass and alleviate tumor pathology. NFZ downregulated IL-6, TNF-α and NF-κb, enhanced angiostatin, decreased tumor VEG and downregulated STAT3 phosphorylation in a dose-dependent manner. It was able to inhibit the growth of solid breast cancer *in vivo*.

*JAK/STAT3 increases breast cancer stem cells and cancer chemotherapy resistance by regulating lipid metabolism.* STAT3 is a direct inflammatory cytokine target (70), and JAK/STAT3 increases breast cancer stem cells and cancer chemotherapy resistance by regulating lipid metabolism. Inhibition of the JAK/STAT3 signaling pathway was able to reduce the characteristics of breast cancer stem cells and the expression of multiple lipid metabolism genes (71-74).

*NFZ targets ALDH1.* Studies have shown that NFZ targets STAT3 and inactivates ALDH1 to inhibit multiple myeloma and melanoma cells, respectively (10,75,76). NFZ can be biologically activated by ALDH, which is highly expressed in certain cancer-initiating cells (ALDH-high stem cells). Although ALDH2 is a direct target of NFZ (75), the biological activity of NFZ against ALDH1 is superior to that of ALDH2 (76). *In vivo*, the drug binds a substrate bag suitable for the ald1a 1/A3 subtype to two cysteine residues at the active site of the enzyme, resulting in oxidation and inactivation of the

enzyme. NFX selectively kills ALDH1-high cancer-initiating cells, which correspond to a high tumorigenic subgroup. In stark contrast, Odero cells were found to be resistant to NFZ. *In vivo* experiments using mice transplanted with A375-L2T melanoma cells showed that the ALDH1-high cancer subpopulation was highly sensitive to NFZ, which completely eradicated the population *in vivo* (77).

*NFZ induces programmed cell necrosis and apoptosis through ERG*. NFZ can activate different regulated cell death pathways (parthanatos and apoptosis) (74-80). NFZ affects multiple transcription factors through the ERG-associated protein-protein interaction network in the MAPK signaling pathway. NFZ can downregulate IL-1, IL-6 and C-C motif chemokine ligand-2 (81). ERG and other transcription factors containing ETS domains work with multiple transcription factors (activator protein-1, nuclear factor of activated T cells, NF-κB) or proteins to regulate downstream gene expression (5,82,83). After NFZ binds to ERG, the spatial conformation of ERG is changed, which makes ERG unable to combine with poly(ADP-ribose) polymerase 1 (PARP1) and other proteins smoothly. Researchers such as Hossain and Bostwick (84) through knocked down ERG (83) confirmed that NFZ blocked the interaction between ERG and PARP1 (84,85). NFZ inhibited the proliferation of transmembrane protease serine:ERG-positive cells by interfering with ERG or ERG-associated TFs. NFZ has a stronger affinity for ERG than STAT3 and ALDH1 (75). NFZ showed a strong inhibitory effect on the growth of ERG-positive prostate cancer cell lines (VCaP, DU145-ERG), but did not inhibit the growth of ERG-negative cell lines (LNCaP, DU145, WPMY) (84). NFZ had stronger inhibitory effects on ERG-overexpressing cell lines. The $IC_{50}$ difference between the DU145-ERG and DU145 vectors was at least 7-fold, and the addition of olapalil reduced the inhibition of DU145-ERG by NFZ by at least 10-fold (82). NFZ upregulated genes associated with DNA repair in prostate cancer VCaP cells (BRCA1, FA complementation group and PARP1), particularly PARP1. PARP1 inhibitors (olapalil) blocked NFZ-mediated growth inhibition of VCaP cells in a dose-dependent manner. An increase in intracellular allograft inflammatory factor occurred, which eventually produced a large number of DNA fragments to induce cell necrosis (86,87).

*NFZ targets USP-21*. USP is an enzyme that catalyzes protein deubiquitination and is involved in biological processes related to metabolic disorders and cancer proliferation. Abnormal USP function has been associated with a variety of diseases, including metabolic dysfunction associated with liver disease and cancer (85). Certain USPs regulate oncogene activity and/or tumor suppressor function, while others influence pathways associated with tumor progression (88). In HepG2 cells, NFZ increased miR-4458 levels and not only inhibited USP-21 and its substrate ATP citrate lyase, but also increased p-AMP kinase α (the downstream functional target of USP-21). Thus, NFZ may be characterized by the fact that in the chemical structure of nifuramide, the oxygen atoms of the nitro group and the oxygen atoms of the carbonyl group exhibit higher electron densities due to their binding within the conjugated system. As a result, these oxygen atoms have the ability to form strong bonds with amino acids with opposite charges. Previous studies have also revealed the anti-proliferative properties of furan-containing compounds (85), while nitrofuran derivatives have shown tumor growth inhibition through p53-dependent mechanisms (89), and the anticancer potential of amide compounds has been similarly confirmed. For instance, decibuprofen and amide derivatives inhibit MCF-7 cell proliferation (90), and these derivatives inhibit the growth of multiple cancer cell lines by interacting with the tyrosine kinase domain of human epidermal growth factor receptor 2 (91). The association of USP-21 (92,93) extends to the stem cell regulation of cancer cells, which activates the Wnt pathway to enhance the stem cell properties of pancreatic cancer cells (94). In addition, USP21 is involved in the deubiquitination of K48-linked ubiquitin chains, stabilizing Nanog, and maintaining the dryness of mouse embryonic stem cells in both *in vivo* and *in vitro* environments (95). In bladder cancer, USP21 expression is elevated and associated with poor prognosis. Notably, it blocks ubiquitination of EZH2, thereby promoting the proliferation and metastasis of bladder cancer cells (96). In non-small cell lung cancer, USP21 promotes tumor cell proliferation, invasion and migration through the YY1/small nucleolar RNA host gene 16 pathway (97). The interaction of USP21 with MAPK kinase 2 stabilizes the latter, ultimately leading to the activation of ERK1/2 and the propagation of carcinogenic signals in liver cancer (98,99).

## 4. Tumor-surrounding environment/immunity

Cell necrosis and downregulation of these inflammatory factors caused by NFZ activation of parthanatos can alter the immune microenvironment and enhance the immune response (81). NFZ can increase the infiltration of CD8+ T cells and decrease the number of M2 macrophages in colorectal cancer; the percentage of M2 macrophages (CD11b+F4/80+CD206+) blank group was 10.2%, the percentage in the 25 mg/kg group (CD11b+F4/80+CD206+) was 8.6% and the percentage in the 50 mg/kg group (CD11b+F4/80+CD206+) was 3.6% (11). NFZ reduced the number of bone marrow-derived suppressor cells in breast cancer cells and colorectal cancer cells, and increased intratumoral CD8+ T-cell infiltration. Importantly, a significant decrease and changes in the number of M2-type macrophages in the tumor were observed in a model of abdominal metastasis (78).

## 5. Drug combinations

STAT3 as the target was able to increase the sensitivity to olaparil (100) (Fig. 2). NFZ reduced cell proliferation *in vitro*, adenovirus combined with oncolytic action was able to enhance the therapeutic effect of STAT3/5 inhibitors on bladder cancer (8) and attenuated salmonella carrying NFZ and programmed cell death 1 (PD-1) small interfering RNA could effectively inhibit the development of colon cancer (99). Combined use can improve the survival rate of mice, stimulate strong anti-tumor immunity and improve the therapeutic effect of anti-tumor immunity. Its mechanisms are mainly involved in immune regulation and apoptosis (101). Induction of PD-1 ligand 1 (PD-L1) weakened the antitumor effects of radiation

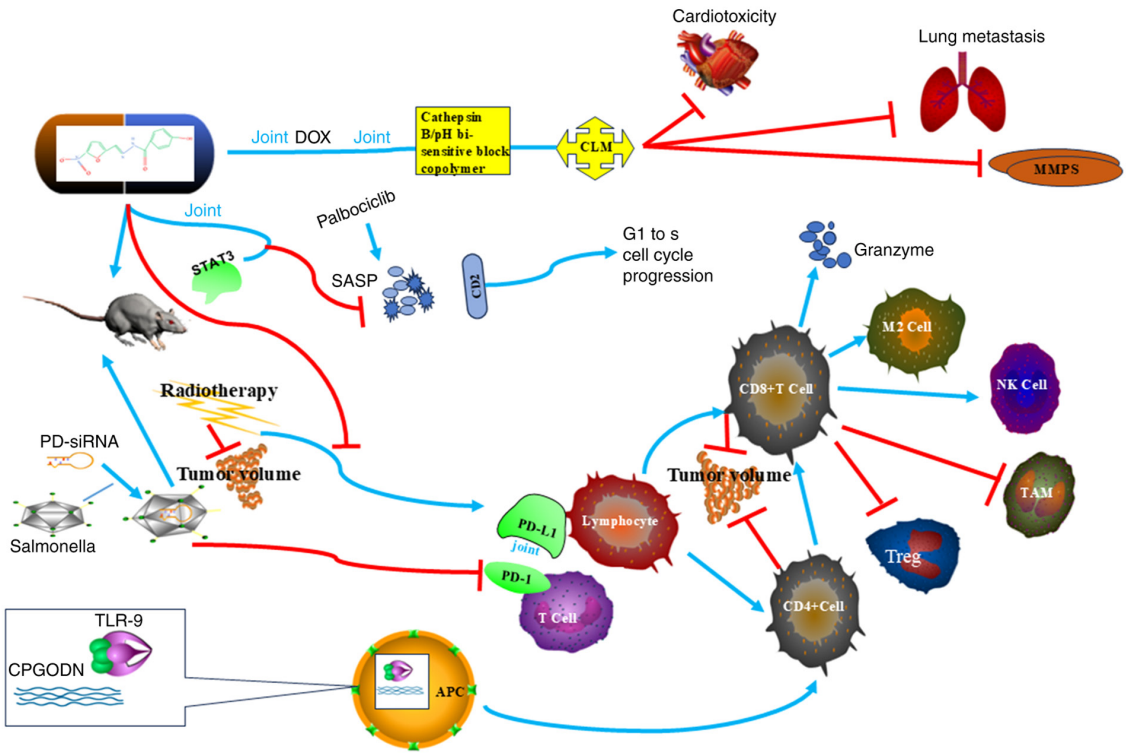


Figure 2. Diagram of the action of NFZ in combination with other treatments. The combination of palbociclib and radiotherapy can increase the efficiency and radiotherapy attenuates the toxicity. NFZ was shown to inhibit tumor growth by inhibiting the phosphorylation of STAT3. CpG ODN as a TLR9 agonist: CpG ODN is absorbed by APCs, with presentation of processed antigenic peptides on MHC II CD4 + Th cells and further activation of CD8 + T cells, which triggers an immune response. Combination with NFZ can enhance the antitumor effect *in vivo* and *in vitro*. The new drug application increases its anti-tumor effect and exerts its multi-protein targeting anti-tumor function. NFZ, nifuroxazide; APC, antigen-presenting cell; MHC, major histocompatibility complex; Th, T-helper; Treg, T-regulatory cell; TAM, tumor-associated macrophage; TLR, Toll-like receptor; siRNA, small interfering RNA; PD-1, programmed cell death 1; PD-L1, PD-1 ligand 1; NK, natural killer; SASP, senescence-associated secretory phenotype; CLM, co-prodrug-loaded micelles; DOX, doxorubicin; ODN, a specific non-methylated CpG dinucleotide sequence.

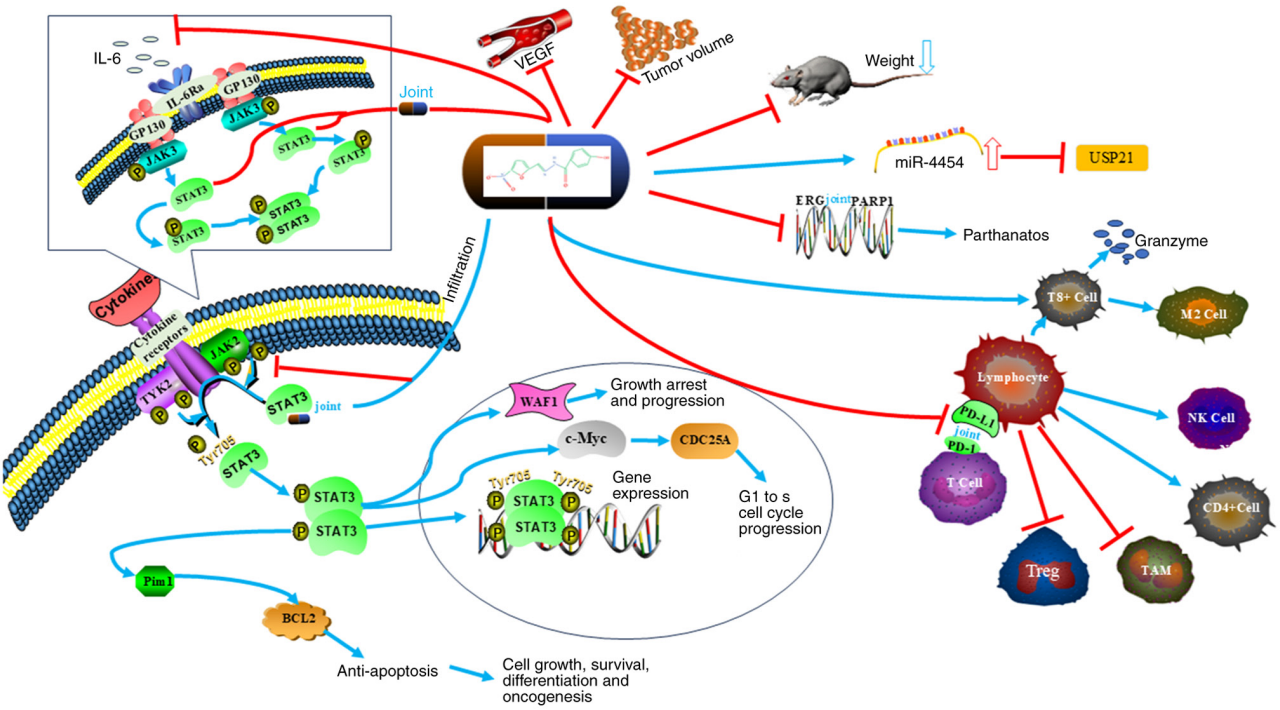


Figure 3. NFZ signaling pathway diagram. NFZ binds to STAT3, ERG and ALDH1, thereby inhibiting tumor proliferation, inhibiting the phosphorylation of JAK2, inhibiting the upstream cytokine IL-6 of STAT3, MAPK and PI3K, and inhibiting the binding of PD-1 and PDL-1, thus acting on tumor immunity. In *in vivo* experiments, it can inhibit VEGF and inhibit tumor vascular hyperplasia. It may reduce the growth of tumor swelling volume in rats and prevent weight loss in rats. NFZ can be targeted against tumors through multiple proteins. NFZ, nifuroxazide; ALDH, aldehyde dehydrogenase; ERG, one of the families of E-twenty-six transcription factors; PD-1, programmed cell death 1; PD-L1, PD-1 ligand 1; NK, natural killer.

Table I. Antitumor effects of different target proteins of nifuroxazide.

| Targets | Target-associated proteins | Activities | Tumor types | (Refs.) |
|---|---|---|---|---|
| JAK2/STAT3 | p-STAT3↓, CDK2↓, SASP2↓, p-RB↓, P21↑, Ki67↓, Cyclin E1↓, Bcl-2↓, Bax↑, CC-3↑, MMP1↓, MMP2↓, MMP9↓, PARP1↑ | G0/G1phase↑; tumor weight↓; pulmonary metastasis↓; cell apoptosis↑; cell invasion↓; migration↓; survival rate↑; lymphocyte infiltration in tumor tissues↑; immune cell response in spleen↑; $CD4^+$ and $CD8^+$ T lymphocytes in the spleen↑ | Osteosarcoma, hepatocarcinoma | (8,21,73) |
| IL-6/STAT3 | Cyclin D1↓, Bcl-2↓, Bcl-xL↑, VEGF↓, VEGFR2↓, MMPs↓, CC-3↑, TNF-α↓, NF-kb, IL-6↓ | Cell apoptosis↑; cell invasion↓; migration↓; survival rate↑; solid breast cancer↓ | Breast cancer | (42,48-51,70,71) |
| ALDH | $ALDH^{High}$↓ | Tumor initiation↓, tumor growth↓ | Multiple myeloma, melanoma, | (9,73) |
| ERG | MAPK↓, IL-1↓, IL-6↓, CCL-2↓, PARP1↑, AIF↑, TMPRSS | Apoptosis↑, necrosis↑ parthanatos ↑ | Prostate cancer | (75) |
| USP-21 | p-AMPKα↑, USP21↓ | Cell proliferation | Hepatocarcinoma, bladder cancer, liver cancer | (84,86) |

CDK, cyclin-dependent kinase; SASP2, small acid-soluble protein 2; p-RB, phosphorylated retinoblastoma protein; P21, p21-activated kinase; Ki67, proliferating cell nuclear antigen Ki-67; Bcl-2, B-cell lymphoma-2; Bax, BCL2-associated X protein; CC-3, cleaved-caspase 3; MMP1, matrix metallopeptidase 1; PARP, poly ADP-ribose polymerase 1; Bcl-xL, B-cell lymphoma-extra large; VEGFR2, vascular endothelial growth factor receptor 2; TNF-α, tumor necrosis factor-α; NF-kb, nuclear factor kappa B; IL-6, Interleukin 6; ALDH, aldehyde dehydrogenase; MAPK, mitogen-activated protein kinase; CCL-2, C-C motif chemokine ligand 2; AIF, apoptosis inducing factor; TMPRSS, transmembrane proteases serine; p-AMPKα, phospho-adenosine monophosphate-activated protein kinase α; USP21, ubiquitin-specific peptidase 21.

therapy. High expression of PD-L1 impaired the anti-tumor function of T lymphocytes and macrophages. In addition, NFZ was able to significantly inhibit radiation-induced upregulation of PD-L1. *In vivo* and *in vitro* experiments have shown that radiotherapy combined with NFZ enhanced T lymphocyte activation and the M1 macrophage ratio, improved the anti-tumor effect of radiotherapy and provided a synergistic treatment strategy for patients with hepatocellular carcinoma (102). *In vivo*, the combination of NFZ with CpG ODN exhibited a therapeutic effect on liver cancer. NFZ inhibited cell proliferation, induced apoptosis and inhibited the migration and invasion of HepG2 cells. The combination of NFZ and CpG ODN had a significant inhibitory effect on tumor growth in tumor-bearing mice and there were almost no side effects. In addition, NFZ combined with CpG ODN treatment significantly induced apoptosis, enhanced the infiltration of CD4+ and CD8+ T lymphocytes and macrophages, and increased the proportion of CD4+ and CD8+ T lymphocytes in the spleen of tumor-bearing mice. The combination of NFZ and CpG ODN provides a new strategy for the treatment of liver cancer (103). A novel lipoprotein-unstable S-2 phosphate antidrug has been synthesized for NFZ, which can be regionally selectively broken down by the abundant membrane enzymes in cancer cells. The cytotoxicity of diazine trioxide nanoparticles self-assembled to <20 nm was determined by the MTT proliferation assay. The results showed that this substance had multiple effects in inhibiting cancer cells compared to proflurazine. The local concentration of the drug was significantly increased, up to 240-fold when assembled into nanoparticles, improving the local action of the drug and enhancing the anti-tumor effect (104). Cathepsin B/pH dual-sensitive block copolymer with a molecular weight of 92 kDa was synthesized to conjugate with doxorubicin (DOX). The copolymer DOX was further loaded with NFX self-assembled co-prodrug-loaded micelles (CLM). CLM showed drug release patterns in response to pH/enzyme dual stimulation and enzyme biodegradation. CLM was shown to reduce the viability of 4T1 murine breast cancer cells and inhibit the migration and *in vitro* culture of invasive mouse breast cancer cells. After intravenous administration of CLM, its nanoscale size and stimulatory reactivity facilitated drug delivery at tumor sites in mice. Both of them had a strong anti-tumor effect and a strong anti-metastasis effect in lung metastasis derived from 4T1. At

the same time, tissue immunofluorescence and immunohistochemical analysis revealed high levels of apoptosis, inhibition of MMP expression and reduction of these all contributed to the inhibition of lung metastasis. The infiltration of MMP pathways and MDSCs associated with the induction of massive metastasis were inhibited in lung metastasis after CLM treatment. In addition, CLM showed excellent biocompatibility with major organs and reduced cardiotoxicity of dox. NFZ also has a vascular protective effect, restoring the oxidation-antioxidant balance, protecting the expression of endothelial nitric oxide synthase in the endothelia and weakening the expression of IL-1β (7,105).

## 6. Safety and no organ toxicity

NFZ has been successfully tested for safety and efficacy in solid tumors in Phase I clinical trials (106), where, after oral administration, the drug can be absorbed and metabolized in the liver, and very low concentrations can be detected in blood and urine. The drug shows almost unique enteral sterility and no systemic antibacterial activity (4). NFZ is well tolerated, but occasionally, side effects occur, manifested by digestive process disturbances and eventual allergic reactions, such as rashes, hives and vascular inflammation (and, in rare cases, severe immune anaphylaxis and anaphylactic shock) (107). There is no carcinogenicity in transgenic mouse models of NFZ mutagenicity (108). Mice treated with NFZ lost significantly less weight than those treated with the carrier, meaning that NFZ reduced weight loss in mice and that NFZ did not show significant toxicity. In the NFZ treatment group, alanine and aspartate aminotransferase values fluctuated within the normal range (109), and NFZ could exert its antitumor effects without significant toxicity. Toxicity evaluation was performed on A375 and B16-F10 model mice to confirm the safety of NFZ after drug therapy, including serological analysis, blood analysis and H&E staining. Serological and hematological analysis did not show any pathological changes and there was no significant change in body weight compared to the control group. In addition, no pathological changes were observed in the heart, liver, spleen and kidney after NFZ treatment (110). NFZ has been shown to inhibit STAT3 phosphorylation by inhibiting JAK2 and Tyk2 family kinases, leading to a decline in myeloma cell viability. However, there was no cytotoxic effect on normal peripheral blood mononuclear cells (8,12,111), which indicates safety to a certain extent in clinical practice.

## 7. Conclusion

Currently, a novel lipoprotein unstable S-2 phosphate, NFZ, has been synthesized, which, when assembled into nanoparticles, significantly increases the local concentration of the drug (240-fold), enhancing the local effect of the drug and enhancing the antitumor effect (108). NFZ is a multi-target anti-tumor drug (Table I), which inhibits cell proliferation, promotes apoptosis and programmed necrosis of tumor cells through the IL-6, STAT3, ALDH1, ERG and UPS-21 pathways, acts on the tumor microenvironment and enhances the anti-tumor effects of T lymphocytes and B lymphocytes (Fig. 3). As a combination drug, NFZ can enhance the effect of radiation therapy, targeted therapy and immunotherapy, and has shown obvious safety in *in vivo* and *in vitro* experiments, thus making it a potential anti-tumor drug (Fig. 2).

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## Authors' contributions

LL wrote the manuscript, searched the literature and prepared the figures. CM was involved in the design of the study and revised the manuscript. DL provided article ideas, modified the figures and revised the manuscript. JJ and RG performed the literature search and critcally revised the manuscript for important intellectual content. Data authentication is not applicable. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## References

1. Carron MCE: Antibacterial nitrofurfuryldene derivatives and methods of using same. US Patent US3290213. Filed July 9, 1962; issued December 6, 1966.
2. B Fernandes M, Gonçalves JE, C Tavares L and Storpirtis S: Caco-2 cells permeability evaluation of nifuroxazide derivatives with potential activity against methicillin-resistant Staphylococcus aureus (MRSA). Drug Dev Ind Pharm 41: 1066-1072, 2015.
3. Bailly C: Toward a repositioning of the antibacterial drug nifuroxazide for cancer treatment. Drug Discov Today 24: 1930-1936, 2019.
4. Nelson EA, Walker SR, Kepich A, Gashin LB, Hideshima T, Ikeda H, Chauhan D, Anderson KC and Frank DA: Nifuroxazide inhibits survival of multiple myeloma cells by directly inhibiting STAT3. Blood 112: 5095-5102, 2008.
5. Li D, Liu L, Li F, Ma C and Ge K: Nifuroxazide induces the apoptosis of human non-small cell lung cancer cells through the endoplasmic reticulum stress PERK signaling pathway. Oncol Lett 25: 248, 2023.
6. Zhao T, Wei P, Zhang C, Zhou S, Liang L, Guo S, Yin Z, Cheng S, Gan Z, Xia Y, *et al*: Nifuroxazide suppresses PD-L1 expression and enhances the efficacy of radiotherapy in hepatocellular carcinoma. Elife 12: RP90911, 2024.
7. Amin FM, Sharawy MH, Amin MN, El-Sherbiny M, Said E, Salem HA and Ibrahim TM: Nifuroxazide mitigates doxorubicin-induced cardiovascular injury: Insight into oxidative/NLRP3/GSDMD-mediated pyroptotic signaling modulation. Life Sci 314: 121311, 2023.

8. Luo Y, Zeng A, Fang A, Song L, Fan C, Zeng C, Ye T, Chen H, Tu C and Xie Y: Nifuroxazide induces apoptosis, inhibits cell migration and invasion in osteosarcoma. Invest New Drugs 37: 1006-1013, 2019.
9. Hindupur SV, Schmid SC, Koch JA, Youssef A, Baur EM, Wang D, Horn T, Slotta-Huspenina J, Gschwend JE, Holm PS and Nawroth R: STAT3/5 inhibitors suppress proliferation in bladder cancer and enhance oncolytic adenovirus therapy. Int J Mol Sci 21: 1106, 2020.
10. Wang X, Shi W, Wang X, Lu JJ, He P, Zhang H and Chen X: Nifuroxazide boosts the anticancer efficacy of palbociclib-induced senescence by dual inhibition of STAT3 and CDK2 in triple-negative breast cancer. Cell Death Discov 9: 355, 2023.
11. El-Sherbiny M, El-Sayed RM, Helal MA, Ibrahiem AT, Elmahdi HS, Eladl MA, Bilay SE, Alshahrani AM, Tawfik MK, Hamed ZE, *et al*: Nifuroxazide mitigates angiogenesis in ehlrich's solid carcinoma: molecular docking, bioinformatic and experimental studies on inhibition of Il-6/Jak2/Stat3 signaling. Molecules 26: 6858, 2021.
12. Zhao T, Jia H, Cheng Q, Xiao Y, Li M, Ren W, Li C, Feng Y, Feng Z, Wang H and Zheng J: Nifuroxazide prompts antitumor immune response of TCL-loaded DC in mice with orthotopically-implanted hepatocarcinoma. Oncol Rep 37: 3405-3414, 2017.
13. Ye TH, Yang FF, Zhu YX, Li YL, Lei Q, Song XJ, Xia Y, Xiong Y, Zhang LD, Wang NY, *et al*: Inhibition of Stat3 signaling pathway by nifuroxazide improves antitumor immunity and impairs colorectal carcinoma metastasis. Cell Death Dis 8: e2534, 2017.
14. Yang F, Hu M, Lei Q, Xia Y, Zhu Y, Song X, Li Y, Jie H, Liu C, Xiong Y, *et al*: Nifuroxazide induces apoptosis and impairs pulmonary metastasis in breast cancer model. Cell Death Dis 6: e1701, 2015.
15. Yu H, Lee H, Herrmann A, Buettner R and Jove R: Revisiting STAT3 signalling in cancer: New and unexpected biological functions. Nat Rev Cancer 14: 736-746, 2014.
16. Huynh J, Etemadi N, Hollande F, Ernst M and Buchert M: The JAK/STAT3 axis: A comprehensive drug target for solid malignancies. Semin Cancer Biol 45: 13-22, 2017.
17. Jung KH, Yoo W, Stevenson HL, Deshpande D, Shen H, Gagea M, Yoo SY, Wang J, Eckols TK, Bharadwaj U, *et al*: Multi-functional effects of a small-molecule STAT3 inhibitor on NASH and HCC in mice. Clin Cancer Res 23: 5537-5546, 2017.
18. Guo C, Yang G, Khun K, Kong X, Levy D, Lee P and Melamed J: Activation of Stat3 in renal tumors. Am J Transl Res 1: 283-290, 2009.
19. Tong M, Wang J, Jiang N, Pan H and Li D: Correlation between p-STAT3 overexpression and prognosis in lung cancer: A systematic review and meta-analysis. PLoS One 12: e0182282, 2017.
20. Takemoto S, Ushijima K, Kawano K, Yamaguchi T, Terada A, Fujiyoshi N, Nishio S, Tsuda N, Ijichi M, Kakuma T, *et al*: Expression of activated signal transducer and activator of transcription-3 predicts poor prognosis in cervical squamous-cell carcinoma. Br J Cancer 101: 967-972, 2009.
21. Chen CL, Cen L, Kohout J, Hutzen B, Chan C, Hsieh FC, Loy A, Huang V, Cheng G and Lin J: Signal transducer and activator of transcription 3 activation is associated with bladder cancer cell growth and survival. Mol Cancer 7: 78, 2008.
22. Hammarén HM, Virtanen AT, Raivola J and Silvennoinen O: The regulation of JAKs in cytokine signaling and its breakdown in disease. Cytokine 118: 48-63, 2019.
23. Jia H, Cui J, Jia X, Zhao J, Feng Y, Zhao P, Zang D, Yu J, Zhao T, Wang H and Xu K: Therapeutic effects of STAT3 inhibition by nifuroxazide on murine acute graft graft-vs.-host disease: Old drug, new use. Mol Med Rep 16: 9480-9486, 2017.
24. Althagafy HS, El-Aziz MKA, Ibrahim IM, Abd-Alhameed EK and Hassanein EHM: Pharmacological updates of nifuroxazide: Promising preclinical effects and the underlying molecular mechanisms. Eur J Pharmacol 951: 175776, 2023.
25. Hasson TS, Said E and Helal MG: Nifuroxazide modulates hepatic expression of LXRs/SR-BI/CES1/CYP7A1 and LDL-R and attenuates experimentally-induced hypercholesterolemia and the associated cardiovascular complications. Life Sci 306: 120790, 2022.
26. Kishimoto T and Ishizaka K: Regulation of antibody response in vitro. X. Biphasic effect of cyclic AMP on the secondary anti-hapten antibody response to anti-immunoglobulin and enhancing soluble factor. J Immunol 116: 534-541, 1976..
27. Darnell JE Jr: Transcription factors as targets for cancer therapy. Nat Rev Cancer 2: 740-749, 2002.
28. Hirano T: Interleukin 6 and its receptor: ten years later. Int Rev Immunol 16: 249-284, 1998.
29. Brumfftt W, Reynolds AV and Hamilton-Miller JM: Letter: Activity of nitrofurantoin and nifuratel against anaerobic gram-negative bacilli. Lancet 1: 460, 1975.
30. Kang S, Narazaki M, Metwally H and Kishimoto T: Historical overview of the interleukin-6 family cytokine. J Exp Med 217: e20190347, 2020.
31. Hirano T and Kishimoto T: Interleukin-6. In: Peptide Growth Factors and Their Receptors I. Sporn MB and Roberts AB (eds). Springer, Berlin, p633, 1990.
32. Heinrich PC, Behrmann I, Haan S, Hermanns HM, Müller-Newen G and Schaper F: Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J 374: 1-20, 2003.
33. Kamimura D, Ishihara K and Hirano T: IL-6 signal transduction and its physiological roles: the signal orchestration model. Rev Physiol Biochem Pharmacol 149: 1-38, 2003.
34. Hasegawa H, Mizoguchi I, Chiba Y, Ohashi M, Xu M and Yoshimoto T: Expanding diversity in molecular structures and functions of the IL-6/IL-12 heterodimeric cytokine family. Front Immunol 7: 479, 2016.
35. Polatti F: Bacterial vaginosis, Atopobium vaginae and nifuratel. Curr Clin Pharmacol 7: 36-40, 2012.
36. Yang L, Wang L, Lin HK, Kan PY, Xie S, Tsai MY, Wang PH, Chen YT and Chang C: Interleukin-6 differentially regulates androgen receptor transactivation via PI3K-Akt, STAT3, and MAPK, three distinct signal pathways in prostate cancer cells. Biochem Biophys Res Commun 305: 462-469, 2003.
37. Yu H, Pardoll D and Jove R: STATs in cancer Inflammation and immunity: A leading role for STAT3. Nat Rev Cancer 9: 798-809, 2009.
38. Catlett-Falcone R, Landowski TH, Oshiro MM, Turkson J, Levitzki A, Savino R, Ciliberto G, Moscinski L, Fernández-Luna JL, Nuñez G, *et al*: Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells. Immunity 10: 105-115, 1999.
39. Zilberstein A, Ruggieri R, Korn JH and Revel M: Structure and expression of cDNA and genes for human interferon-beta-2, a distinct species inducible by growth-stimulatory cytokines. EMBO J 5: 2529-2537, 1986.
40. Haegeman G, Content J, Volckaert G, Derynck R, Tavernier J and Fier W: Structural analysis of the sequence coding for an inducible 26-kDa protein in human fibroblasts. Eur J Biochem 159: 625-632, 1986.
41. Kubo M, Hanada T and Yoshimura A: Suppressors of cytokine signaling and immunity. Nat Immunol 4: 1169-1176, 2003.
42. Zhong Z, Wen Z and Darnell JE Jr: Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. Science 264: 95-98, 1994.
43. Lieblein JC, Ball S, Hutzen B, Sasser AK, Lin HJ, Huang TH, Hall BM and Lin J: STAT3 can be activated through paracrine signaling in breast epithelial cells. BMC Cancer 8: 302, 2008.
44. Chang Q, Bournazou E, Sansone P, Berishaj M, Gao SP, Daly L, Wels J, Theilen T, Granitto S, Zhang X, *et al*: The IL6/JAK/Stat3 feed-forward loop drives tumorigenesis and metastasis. Neoplasia 15: 848-862, 2013.
45. Fukada T, Hibi M, Yamanaka Y, Takahashi-Tezuka M, Fujitani Y, Yamaguchi T, Nakajima K and Hirano T: Two signals are necessary for cell proliferation induced by a cytokine receptorGp130: Involvementof STAT3 inAnti-apoptosis. Immunity 5: 449-460, 1996.
46. Leslie K, Lang C, Devgan G, Azare J, Berishaj M, Gerald W, Kim YB, Paz K, Darnell JE, Albanese C, *et al*: Cyclin D1 is transcriptionally regulated by and required for transformation by activated signal transducer and activator of transcription 3. Cancer Res 66: 2544-2552, 2006.
47. Burke WM, Jin X, Lin HJ, Huang M, Liu R, Reynolds RK and Lin J: Inhibition of constitutively active stat3 suppresses growth of human ovarian and breast cancer cells. Oncogene 20: 7925-7934, 2001.
48. Lee HT, Xue J, Chou PC, Zhou A, Yang P, Conrad CA, Aldape KD, Priebe W, Patterson C, Sawaya R, *et al*: Stat3 orchestrates interaction between endothelial and tumor cells and inhibition of stat3 suppresses brain metastasis of breast cancer cells. Oncotarget 6: 10016-10029, 2015.
49. Vageli DP, Doukas PG, Siametis A and Judson BL: Targeting STAT3 prevents bile reflux-induced oncogenic molecular events linked to hypopharyngeal carcinogenesis. J Cell Mol Med 26: 75-87, 2022.
50. Manore SG, Doheny DL, Wong GL and Lo HW: IL-6/JAK/STAT3 signaling in breast cancer metastasis: Biology and treatment. Front Oncol 12: 866014, 2022.

51. Hirano T, Yasukawa K, Harada H, Taga T, Watanabe Y, Matsuda T, Kashiwamura S, Nakajima K, Koyama K, Iwamatsu A, *et al*: Complementary DNA for a novel human interleukin (BSF-2) that induces B lymphocytes to produce immunoglobulin. Nature 324: 73-76, 1986.
52. Gauldie J, Richards C, Harnish D, Lansdorp P and Baumann H: Interferon beta 2/B-Cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. Proc Natl Acad Sci USA 84: 7251-7255, 1987.
53. Brakenhoff JP, de Groot ER, Evers RF, Pannekoek H and Aarden LA: molecular cloning and expression of hybridoma growth factor in escherichia coli. J Immunol 139: 4116-4121, 1987.
54. Lo HW, Hsu SC, Xia W, Cao X, Shih JY, Wei Y, Abbruzzese JL, Hortobagyi GN and Hung MC: Epidermal growth factor receptor cooperates with signal transducer and activator of transcription 3 to induce epithelial-mesenchymal transition in cancer cells via up-regulation of TWIST gene expression. Cancer Res 67: 9066-9076, 2007.
55. Niu G, Wright KL, Huang M, Song L, Haura E, Turkson J, Zhang S, Wang T, Sinibaldi D, Coppola D, *et al*: Constitutive Stat3 Activity Up-Regulates VEGF expression and tumor angiogenesis. Oncogene 21: 2000-2008, 2002.
56. Kortylewski M and Yu H: Role of stat3 in suppressing anti-tumor immunity. Curr Opin Immunol 20: 228-233, 2008.
57. Wang T, Niu G, Kortylewski M, Burdelya L, Shain K, Zhang S, Bhattacharya R, Gabrilovich D, Heller R, Coppola D, *et al*: Regulation of the innate and adaptive immune responses by stat-3 signaling in tumor cells. Nat Med 10: 48-54, 2004.
58. Carpenter RL and Lo HW: STAT3 target genes relevant to human cancers. Cancers (Basel) 6: 897-925, 2014.
59. Alvarez JV, Febbo PG, Ramaswamy S, Loda M, Richardson A and Frank DA: Identification of a genetic signature of activated signal transducer and activator of transcription 3 in human tumors. Cancer Res 65: 5054-5062, 2005.
60. Dechow TN, Pedranzini L, Leitch A, Leslie K, Gerald WL, Linkov I and Bromberg JF: Requirement of matrix metalloproteinase-9 for the transformation of human mammary epithelial cells by stat3-C. Proc Natl Acad Sci USA 101: 10602-10607, 2004.
61. Kujawski M, Kortylewski M, Lee H, Herrmann A, Kay H and Yu H: Stat3 mediates myeloid cell-dependent tumor angiogenesis in mice. J Clin Invest 118: 3367-3377, 2008.
62. Jiang M, Chen J, Zhang W, Zhang R, Ye Y, Liu P, Yu W, Wei F, Ren X and Yu J: Interleukin-6 transsignaling pathway promotes immunosuppressive myeloid-derived suppressor cells via suppression of suppressor of cytokine signaling 3 in breast cancer. Front Immunol 8: 1840, 2017.
63. Sun Z, Yao Z, Liu S, Tang H and Yan X: An oligonucleotide decoy for stat3 activates the immune response of macrophages to breast cancer. Immunobiology 211: 199-209, 2006.
64. Jones LM, Broz ML, Ranger JJ, Ozcelik J, Ahn R, Zuo D, Ursini-Siegel J, Hallett MT, Krummel M and Muller WJ: STAT3 establishes an immunosuppressive microenvironment during the early stages of breast carcinogenesis to promote tumor growth and metastasis. Cancer Res 76: 1416-1428, 2016.
65. Iliopoulos D, Hirsch HA and Struhl K: An epigenetic switch involving NF-KappaB, Lin28, Let-7 MicroRNA, and IL6 links Inflammation to cell transformation. Cell 139: 693-706, 2009.
66. Barbieri I, Pensa S, Pannellini T, Quaglino E, Maritano D, Demaria M, Voster A, Turkson J, Cavallo F, Watson CJ, *et al*: Constitutively active stat3 enhances neu-mediated migration and metastasis in mammary tumors via upregulation of Cten. Cancer Res 70: 2558-2567, 2010.
67. Yu H, Kortylewski M and Pardoll D: Crosstalk between cancer and immune cells: Role of STAT3 in the tumour microenvironment. Nat Rev Immunol 7: 41-51, 2007.
68. Wei D, Le X, Zheng L, Wang L, Frey JA, Gao AC, Peng Z, Huang S, Xiong HQ, Abbruzzese J and Xie K: Stat3 activation regulates the expression of vascular endothelial growth factor and human pancreatic cancer angiogenesis and metastasis. Oncogene 22: 319-329, 2003.
69. Chen RY, Yen CJ, Liu YW, Guo CG, Weng CY, Lai CH, Wang JM, Lin YJ and Hung LY: CPAP promotes angiogenesis and metastasis by enhancing STAT3 activity. Cell Death Differ 27: 1259-1273, 2020.
70. Johnson DE, O'Keefe RA and Grandis JR: Targeting the IL-6/JAK/STAT3 signalling axis in cancer. Nat Rev Clin Oncol 15: 234-248, 2018.
71. Khatib A, Solaimuthu B, Ben Yosef M, Abu Rmaileh A, Tanna M, Oren G, Schlesinger Frisch M, Axelrod JH, Lichtenstein M and Shaul YD: The glutathione peroxidase 8 (GPX8)/IL-6/STAT3 axis is essential in maintaining an aggressive breast cancer phenotype. Proc Natl Acad Sci USA 117: 21420-21431, 2020.
72. Siersbæk R, Scabia V, Nagarajan S, Chernukhin I, Papachristou EK, Broome R, Johnston SJ, Joosten SEP, Green AR, Kumar S, *et al*: IL6/STAT3 signaling hijacks estrogen receptor α enhancers to drive breast cancer metastasis. Cancer Cell 38: 412-423.e9, 2020.
73. Liu JY, Zhang YC, Song LN, Zhang L, Yang FY, Zhu XR, Cheng ZQ, Cao X and Yang JK: Nifuroxazide ameliorates lipid and glucose metabolism in palmitate-induced HepG2 cells. RSC Adv 9: 39394-39404, 2019.
74. Wang T, Fahrmann JF, Lee H, Li YJ, Tripathi SC, Yue C, Zhang C, Lifshitz V, Song J, Yuan Y, *et al*: JAK/STAT3-regulated fatty acid β-oxidation is critical for breast cancer stem cell self-renewal and chemoresistance. Cell Metab 27: 136-150.e5, 2018.
75. Sarvi S, Crispin R, Lu Y, Zeng L, Hurley TD, Houston DR, von Kriegsheim A, Chen CH, Mochly-Rosen D, Ranzani M, *et al*: ALDH1 bioactivates nifuroxazide to eradicate $ALDH^{High}$ melanoma-initiating cells. Cell Chem Biol 25: 1456-1469.e6, 2018.
76. Zhou L, Ishizaki H, Spitzer M, Taylor KL, Temperley ND, Johnson SL, Brear P, Gautier P, Zeng Z, Mitchell A, *et al*: ALDH2 mediates 5-nitrofuran activity in multiple species. Cell Chem Biol 27: 1452, 2020.
77. Ismail IH and Hendzel MJ: The gamma-H2A.X: Is it just a surrogate marker of double-strand breaks or much more? Environ Mol Mutagen 49: 73-82, 2008.
78. Genin M, Clement F, Fattaccioli A, Raes M and Michiels C: M1 and M2 macrophages derived from THP-1 cells differentially modulate the response of cancer cells to etoposide. BMC Cancer 15: 577, 2015.
79. Sizemore GM, Pitarresi JR, Balakrishnan S and Ostrowski MC: The ETS family of oncogenic transcription factorsin solid tumors. Nat Rev Cancer 17: 337-351, 2017.
80. Verger A, Buisine E, Carrere S, Wintjens R, Flourens A, Coll J, Stéhelin D and Duterque-Coquillaud M: Identification of amino acid residues in the ETS transcription factor Erg that mediate Erg-Jun/Fos-DNA ternary complex formation. J Biol Chem 276: 17181-17189, 2001.
81. Bassuk AG, Anandappa RT and Leiden JM: Physical interactions between Ets and NF-kappaB/NFAT proteins play an important role in their cooperative activation of the human immunodeficiency virus enhancer in T cells. J Virol 71: 3563-3573, 1997.
82. Li C, Zhang J, Wu Q, Kumar A, Pan G and Kelvin DJ: Nifuroxazide activates the parthanatos to overcome TMPRSS2: ERG fusion-positive prostate cancer. Mol Cancer Ther 22: 306-316, 2023.
83. Cobrinik D: Pocket proteins and cell cycle control. Oncogene 24: 2796-2809, 2005.
84. Kron KJ, Murison A, Zhou S, Huang V, Yamaguchi TN, Shiah YJ, Fraser M, van der Kwast T, Boutros PC, Bristow RG and Lupien M: TMPRSS2-ERG fusion co-opts master transcription factors and activates NOTCH signaling in primary prostate cancer. Nat Genet 49: 1336-1345, 2017.
85. Wang Y, An R, Umanah GK, Park H, Nambiar K, Eacker SM, Kim B, Bao L, Harraz MM, Chang C, *et al*: A nuclease that mediates cell death induced by DNA damage and poly(ADP-ribose) polymerase-1. Science 354: aad6872, 2016.
86. Tak J, Nguyen TK, Lee K, Kim SG and Ahn HC: Utilizing machine learning to identify nifuroxazide as an inhibitor of ubiquitin-specific protease 21 in a drug repositioning strategy. Biomed Pharmacother 174: 116459, 2024.
87. Harrigan JA, Jacq X, Martin NM and Jackson SP: Deubiquitylating enzymes and drug discovery: Emerging opportunities. Nat Rev Drug Discov 17: 57-78, 2018.
88. Kanan D, Kanan T, Dogan B, Orhan MD, Avsar T and Durdagi S: An integrated in silico approach and in vitro study for the discovery of small-molecule USP7 inhibitors as potential cancer therapies. ChemMedChem 16: 555-567, 2021.
89. Saito Y, Kishimoto M, Yoshizawa Y and Kawaii S: Synthesis and structure-activity relationship studies of furan-ring fused chalcones as antiproliferative agents. Anticancer Res 35: 811-817, 2015.
90. Al Koussa HK, Abrahamian CF, Elzahhar PM, Serie MA, Belal A and El-Yazbi AF: A novel series of nitrofuran derivatives produces an anti-tumor effect via a p53-dependent mechanism. FASEB J 34: 1, 2020.

91. Ashraf Z, Mahmood T, Hassan M, Afzal S, Rafique H, Afzal K and Latip J: Dexibuprofen amide derivatives as potential anticancer agents: Synthesis, in silico docking, bioevaluation, and molecular dynamic simulation. Drug Des Devel Ther 13: 1643-1657, 2019.
92. Guo Q, Shi D, Lin L, Li H, Wei Y, Li B and Wu D: De-ubiquitinating enzymes USP21 regulate MAPK1 expression by binding to transcription factor GATA3 to regulate tumor growth and cell stemness of gastric cancer. Front Cell Dev Biol 9: 641981, 2021.
93. Zhang Q, Chen Z, Tang Q, Wang Z, Lu J, You Y and Wang H: USP21 promotes self-renewal and tumorigenicity of mesenchymal glioblastoma stem cells by deubiquitinating and stabilizing FOXD1. Cell Death Dis 13: 712, 2022.
94. Ali SH, Osmaniye D, Sağlık BN, Levent S, Özkay Y and Kaplancıklı ZA: Design, synthesis, and molecular docking studies of novel quinoxaline derivatives as anticancer agents. Chem Biol Drug Des 102: 303-315, 2023.
95. Hou P, Ma X, Zhang Q, Wu CJ, Liao W, Li J, Wang H, Zhao J, Zhou X, Guan C, *et al*: USP21 deubiquitinase promotes pancreas cancer cell stemness via Wnt pathway activation. Genes Dev 33: 1361-1366, 2019.
96. Liu X, Yao Y, Ding H, Han C, Chen Y, Zhang Y, Wang C, Zhang X, Zhang Y, Zhai Y, *et al*: USP21 deubiquitylates Nanog to regulate protein stability and stem cell pluripotency. Signal Transduct Target Ther 1: 16024, 2016.
97. Chen Y, Zhou B and Chen D: USP21 promotes cell proliferation and metastasis through suppressing EZH2 ubiquitination in bladder carcinoma. Onco Targets Ther 10: 681-689, 2017.
98. Xu P, Xiao H, Yang Q, Hu R, Jiang L, Bi R, Jiang X, Wang L, Mei J, Ding F and Huang J: The USP21/YY1/SNHG16 axis contributes to tumor proliferation, migration, and invasion of non-small-cell lung cancer. Exp Mol Med 52: 41-55, 2020.
99. Li W, Cui K, Prochownik EV and Li Y: The deubiquitinase USP21 stabilizes MEK2 to promote tumor growth. Cell Death Dis 9: 482, 2018.
100. Hassanein EHM, Abdel-Reheim MA, Althagafy HS, Hemeda MS, Gad RA and Abdel-Sattar AR: Nifuroxazide attenuates indomethacin-induced renal injury by upregulating Nrf2/HO-1 and cytoglobin and suppressing NADPH-oxidase, NF-κB, and JAK-1/STAT3 signals. Naunyn Schmiedebergs Arch Pharmacol 397: 3985-3994, 2024.
101. Mcintosh MT, Koganti S, Boatwright JL, Li X, Spadaro SV, Brantly AC, Ayers JB, Perez RD, Burton EM, Burgula S, *et al*: STAT3 imparts BRCAness by impairing homologous recombination repair in Epstein-Barr virus-transformed B lymphocytes. PLoS Pathog 16: e1008849, 2020.
102. Ettner NM, Vijayaraghavan S, Durak MG, Bui T, Kohansal M, Ha MJ, Liu B, Rao X, Wang J, Yi M, *et al*: Combined inhibition of STAT3 and DNA repair in palbociclib-resistant ER-positive breast cancer. Clin Cancer Res 25: 3996-4013, 2019.
103. Wang Y, Liu W, Liu M, Wang H, Zhou L, Chen J, Sun H, Wei X, Fan M, Yang M, *et al*: Nifuroxazide in combination with CpG ODN exerts greater efficacy against hepatocellular carcinoma. Int Immunopharmacol 108: 108911, 2022.
104. Misra SK, Wu Z, Ostadhossein F, Ye M, Boateng K, Schulten K, Tajkhorshid E and Pan D: Pro-nifuroxazide self-assembly leads to triggerable nanomedicine for anti-cancer therapy. ACS Appl Mater Interfaces 11: 18074-18089, 2019.
105. Luo L, Xu F, Peng H, Luo Y, Tian X, Battaglia G, Zhang H, Gong Q, Gu Z and Luo K: Stimuli-responsive polymeric prodrug-based nanomedicine delivering nifuroxazide and doxorubicin against primary breast cancer and pulmonary metastasis. J Control Release 318: 124-135, 2020.
106. Zhao T, Feng Y, Guo M, Zhang C, Wu Q, Chen J, Guo S, Liu S, Zhou Q, Wang Z, *et al*: Combination of attenuated Salmonella carrying PD-1 siRNA with nifuroxazide for colon cancer therapy. J Cell Biochem 121: 1973-1985, 2020.
107. Wong ALA, Hirpara JL, Pervaiz S, Eu JQ, Sethi G and Goh BC: Do STAT3 inhibitors have potential in the future for cancer therapy? Expert Opin Investig Drugs 26: 883-887, 2017.
108. Shindano A, Marot L and Geubel AP: Nifuroxazide-induced acute pancreatitis: A new side-effect for an old drug? Acta Gastroenterol Belg 70: 32-33, 2007.
109. Quillardet P, Arrault X, Michel V and Touati E: Organ-targeted mutagenicity of nitrofurantoin in Big Blue transgenic mice. Mutagenesis 21: 305-311, 2006.
110. Mazzaccara C, Labruna G, Cito G, Scarfò M, De Felice M, Pastore L and Sacchetti L: Age-related reference intervals of the main biochemical and hematological parameters in C57BL/6J, 129SV/EV and C3H/HeJ mouse strains. PLoS One 3: e3772, 2008.
111. Cipolla BG, Havouis R and Moulinoux JP: Polyamine contents in current foods: A basis for polyamine reduced diet and a study of its long term observance and tolerance in prostate carcinoma patients. Amino Acids 33: 203-212, 2007.