

Perspective

Ritonavir and DNA Damage: A New Perspective on an Old Drug

Silvia Pomella ^{1,2,*} , Erika Ferraro ² , Francesco Marampon ³  and Giovanni Barillari ¹ ¹ Department of Clinical Sciences and Translational Medicine, University of Rome Tor Vergata, 00133 Rome, Italy; barillar@uniroma2.it² Department of Hematology and Oncology, Cell and Gene Therapy, Bambino Gesù Children's Hospital, IRCCS, 00146 Rome, Italy; erika.ferraro@opbg.net³ Department of Radiological Oncological and Pathological Sciences, Sapienza University of Rome, 00185 Rome, Italy; francesco.marampon@uniroma1.it

* Correspondence: silvia.pomella@uniroma2.it or silvia.pomella@opbg.net

Abstract

Ritonavir (RTV), an effective aspartyl protease inhibitor, was originally developed to counter the replication of human immune deficiency virus and then employed as a pharmacokinetic enhancer in antiretroviral therapy. Yet unexpectedly, RTV exerted antitumor effects that added to its antiviral action, as it impacted the migration, invasion, oxidative stress, and proteasome function of human tumor cells. More recently, RTV was shown to directly inhibit DNA repair enzymes, thereby enhancing radiosensitivity and synergizing with chemotherapeutics across multiple cancer models. However, RTV induced oxidative stress and DNA damage also in non-tumor cells, including the reproductive ones. This duality highlights both the possibility of RTV anticancer use and the concern for its safety. In this Perspective, we propose the repurposing of RTV as a novel tool to potentiate DNA-damage-based antitumor therapies such as radiotherapy and/or chemotherapy. At the same time, we underscore the need for a careful assessment of RTV side effects.

Keywords: Ritonavir; HIV protease inhibitors; DNA damage; radiosensitizer; chemosensitizer; drug repurpose



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1. Introduction

The aspartyl protease inhibitor Ritonavir (RTV) was introduced in 1996 as a cornerstone of combination antiretroviral therapy for human immunodeficiency virus (HIV) [1,2]. Although RTV was initially prescribed as an inhibitor of HIV protease (Figure 1), its clinical use shifted after recognition of its potent repression of Cytochrome P450 3A4 (CYP3A4) activity [3]. This property allowed for the use of RTV as a pharmacokinetic enhancer, prolonging the half-life of combined antiviral agents and then boosting the antiretroviral therapy [3]. Beyond these canonical activities of RTV, preclinical studies revealed that this drug exerts unpredicted pleiotropic effects that also occur in the absence of HIV-1 infection: these included the modulation of oxidative stress [4], mitochondrial activity [5], and proteasomal function [6]. Initially, these observations raised concerns regarding genotoxicity and potential long-term risk [7]. More recently, these same properties have been reinterpreted as opportunities for therapeutic repurposing [8]. By promoting DNA damage and disrupting repair and survival pathways (Figure 1), RTV acts as a potential radio- and chemosensitizer in cancer therapy [9–12].

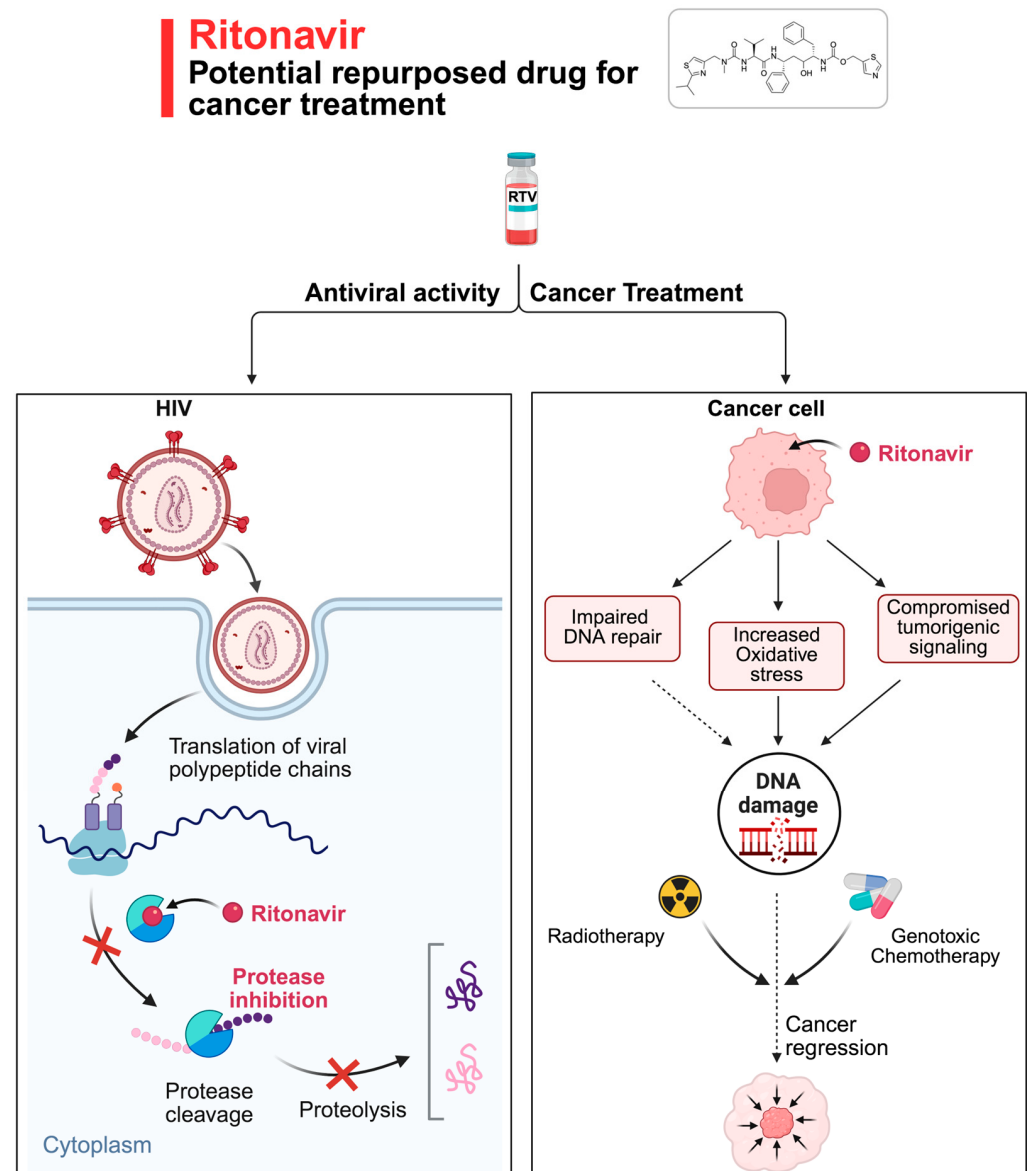


Figure 1. Schematic representation of RTV activities. The canonical antiviral activity of RTV as a peptidomimetic HIV protease inhibitor (left) and its repurposed antitumor activity (right). In cancer cells, RTV impairs DNA repair, modulates oxidative stress, and disrupts pathways essential for survival. The resulting accumulation of DNA damage sensitizes cancer cells to radiotherapy and/or genotoxic chemotherapy, providing a strong rationale for combination therapies. Experimentally validated processes (solid arrows) and proposed mechanisms (dashed arrows). Created in BioRender (<https://app.biorender.com/> accessed on 5 September 2025).

This Perspective traces RTV's transition from antiviral to pharmacokinetic enhancer and potential anticancer agent, emphasizing drug repurposing as facilitating new therapeutic opportunities. We highlight current advances in RTV repurposing for oncology, focusing on the RTV capability of countering epithelial-to-mesenchymal transition (EMT), phosphoinositide 3 kinase/protein kinase B (AKT) signaling, and DNA repair. In addition, we discuss RTV synergy with radio- and chemotherapy, while considering its toxicity for normal, non-tumor cells.

2. From Antiviral to Anticancer Drug: Drug Repurposing

The anticancer potential of RTV emerged from observations that, independently from their antiviral activity, first-generation HIV protease inhibitors exert off-target cytotoxicity

on Kaposi's Sarcoma, the most common HIV-associated malignancy [13]. Results from additional preclinical and clinical work have indicated that RTV is effective against several types of cancer, among which are breast [10], cervical [14,15], pancreatic [16], and head and neck carcinomas [17]. Particularly, RTV has been shown to inhibit the activity of extracellular matrix-degrading, pro-invasive enzymes [11,14,15], perturb tumor metabolism by inhibiting glucose uptake [18], alter protein stability by impairing proteasome function [15,19], disrupt multiple pathways critical for cancer cell survival like AKT [9,15], and counter CYP3A4 activity [20] and NF κ B signaling [21]. Moreover, recent computational docking simulations have shown that RTV binds stably to oncogenic proteins, including PDGFR and ALK, often with higher affinity than canonical inhibitors [22]. By targeting these molecular pathways, RTV could reduce the adaptive capacity of cancer cells, thus enhancing the efficacy of first-line therapies, laying the groundwork for rational combination strategies. These observations support RTV as a clinically accessible, multi-modal anticancer agent, ideal for upcoming repurposing trials.

3. RTV and DNA Repair: Lessons from Recent Studies

Late investigations have strengthened the link between RTV treatment and modulation of DNA damage. In particular, a recent study has demonstrated that RTV inhibits the human DNA repair enzyme ALKBH2, which is responsible for repairing alkylated DNA bases by removing methylated DNA adducts [12]. By impairing this repair, RTV sensitizes prostate cancer cells to alkylating agents, paving the way for further combination with chemotherapies that rely on such damage.

The ability of RTV to cooperate with existing chemotherapeutics has been further described in cervical cancer models, where RTV has been shown to synergize with cisplatin at promoting tumor cell death [22]. Such findings suggest that RTV can complement platinum-based therapeutic approach, one of the most widely used chemotherapeutic classes in oncology, potentially enabling lower doses and mitigating systemic toxicity.

Evidence is also emerging for RTV capacity to enhance radiosensitivity. Specifically, our recent work has indicated that therapeutic doses of RTV enhance the radiosensitivity of oral squamous cell carcinoma cells by countering two key drivers of radioresistance, namely the EMT and the activation (phosphorylation) of the AKT signaling molecule [11]. It is likely that such phenotypic reprogramming of carcinoma cells promoted by RTV may underlie the response of human tumor cells to that antiviral drug and the eventual success of the latter in the treatment of cancer patients.

Investigations on the toxicological profile of RTV have further emphasized the impact that this antiviral drug has on DNA integrity and cellular stress pathways. As a matter of fact, eco-genotoxicity research highlights RTV persistence as well as its genotoxic effects in aquatic organisms [23]. Additional studies have revealed that high concentration of RTV (500 μ M), that are far above the therapeutic ones, compromises the homeostasis and reproductive function of murine Sertoli and Leydig cells by inducing both the production of reactive oxygen species (ROS) and DNA damage accumulation [24]. While these findings underline the need for caution, they also provide consistent evidence that RTV is active at the level of DNA damage and repair, biological properties that could be redirected towards therapeutic benefit in oncology.

4. DNA Damage Response: A Key Pathway in Cancer Therapy

In the context of anticancer therapy, the efficacy of first-line treatments like chemotherapy and radiotherapy rely on the capacity to induce DNA modifications or DNA breaks in tumor cells [25]. In this context, however, one should consider that DNA is continuously exposed to both endogenous (i.e., ROS and lipid peroxidation) and exogenous

(i.e., ultraviolet light, ionizing radiation) damaging agents [26], and that most of the lesions are efficiently repaired through the DNA damage response (DDR) pathway. The latter senses DNA lesions, halts cell-cycle progression, and activates repair mechanisms with the final aim to preserve genome integrity [27]. Nonetheless, whenever an unfeasible repair occurs, DDR can drive cells into apoptosis or senescence [28].

The recognition of DNA lesions is mediated by DDR sensors that, by detecting double-strand (MRN complex) or single-strand (RPA) breaks, activate upstream kinases (ATM, ATR, and DNA-PKcs) and downstream mediators (H2AX, CHK1, and CHK2) triggering signaling cascades that arrest the cell cycle and recruit repair proteins [29]. Specific repair pathways are activated based on the nature of the damage: base excision repair corrects oxidized or alkylated bases, nucleotide excision repair removes bulky adducts, mismatch repair resolves replication errors, and double-strand breaks are processed by non-homologous end joining or homologous recombination [25].

Interestingly, mutations or functional impairments in DDR genes are hallmarks of many cancers, fostering genomic instability and influencing therapeutic response [30,31]. Indeed, defective homologous recombination pathway (i.e., BRCA1/2 mutations) renders cancer cells sensitive to PARP inhibition due to synthetic lethality [32]. In contrast, overactivation of repair enzymes, including ALKBH2, or checkpoint signaling confers resistance to radiation and genotoxic chemotherapy [12,33,34].

In this context, the ability of RTV to modulate DDR is particularly relevant. By targeting ALKBH2 [12,33,34] and inducing oxidative stress [35], RTV could amplify DNA lesions beyond the compensatory capacity of tumor cells.

Notably, RTV exerts a multimodal activity that differs from canonical DDR inhibitors such as PARP or ATR inhibitors. Indeed, RTV does not cause synthetic lethality *per se* but enhances DNA-damage load and proteostatic stress. This broader mechanism could complement DDR-targeting agents, potentially leading to synergistic effects in tumors with defective repair mechanisms. However, given that RTV is a strong CYP3A4 inhibitor, pharmacokinetic interactions should be carefully considered; for instance, itraconazole, another potent CYP3A4 inhibitor, has been shown to increase Olaparib AUC (Area Under the Curve) by 2.7-fold, and a similar effect can be expected with RTV [36]. These aspects warrant further preclinical investigation to define the therapeutic potential and safety of RTV-based combination strategies.

5. Repurposing RTV as a Radiosensitizer and Chemosensitizer

By impairing DNA repair and increasing genomic stress, RTV may enhance the effects of radio- and chemotherapy. Mechanistically, RTV appears to operate through multiple and converging pathways, facilitating enhancement of ROS production [4], inhibition of DNA repair enzymes [12], phenotypic reprogramming that restores sensitivity to radiation [11] and chemotherapeutics [22], and a severe alteration of tumor metabolism [5] and proteasomal function [6]. RTV has also been shown to impair mitochondrial function and oxidative metabolism, increasing ROS production and causing oxidative damage to both nuclear and mitochondrial DNA [35,37], thereby contributing to its overall genotoxic potential.

Indeed, RTV induces DNA damage through multiple, partially overlapping mechanisms. First, RTV directly inhibits DNA repair enzymes such as ALKBH2, impairing the correction of alkylated DNA bases [12,33,34]. Second, RTV increases intracellular oxidative stress [4], leading to secondary DNA lesions. Finally, RTV modulates stress-response pathways, including AKT signaling [11], mitochondria-mediated apoptosis through activation of caspase-9/7 and PARP-1 [5], and proteasome function [6], which may indirectly affect DNA integrity and repair capacity.

These effects are dose-dependent: higher concentrations of RTV amplify oxidative stress [24], whereas clinically relevant concentrations (10–20 μM), corresponding to peak plasma levels observed in patients [14,15], induce moderate but significant DNA damaging effects in cancer cells (Table 1).

Table 1. Summary of the in vitro and in vivo studies supporting the activity of RTV in tumor models.

Refs.	Tumor Type	Cell Lines	In Vitro Range	In Vivo Range	Outcome
[9]	Ovarian cancer	MDAH-2774, SKOV-3	5–25 μM	NA	Inhibition of AKT, inhibition of migration, invasion, and induction of apoptosis
[10]	Breast cancer	MCF7, T47D, MDA-MB-231, MDA-MB-436	15–45 μM	40 mg/kg daily for 52 days, IP	Inhibition of AKT, inhibition of migration, reduction in tumor growth, induction of apoptosis, S-phase cell-cycle arrest
[11]	Oral squamous cell carcinoma	NOK, SSC-25, Detroit 562	10 μM	NA	Inhibition of EMT and sensitization to IR
[12]	Prostate cancer	PC3 ALKBH3-KD	1–50 μM	NA	Sensitization to alkylating agent methylmethane sulfonate
[13]	Kaposi sarcoma	KSIMM, HUVEC	3–30 μM	30 mg/kg daily for 15 days, IP	Inhibition of NF- κB , reduction in cell adhesion and tumor growth
[14]	Cervical intraepithelial neoplasia	W12, CIN612-7E	10 μM	NA	Inhibition of AKT and down-regulation MMP-9
[15]	Cervical intraepithelial neoplasia	CIN612-7E, CIN612-9E, NHEK, SiHa, CaSki	10 μM	NA	Inhibition of invasion and down-regulation of MMP-2 and MMP-9
[16]	Pancreatic cancer	BxPC-3, MIA PaCa-2, PANC-1	5–30 μM	NA	Inhibition of AKT, inhibition of invasion, cell-cycle arrest and induction of cell death
[17]	Head and neck carcinoma	HEP-2	20–2000 μM	8 mg twice a day, 5 days a week, OG	Sensitization to IR and enhancement of IR-induced apoptosis
[18]	Chronic lymphocytic leukemia	Primary patient cells	20 μM	NA	Inhibition of GLUT4 and inhibition of glucose uptake
[19]	Fibrosarcoma, Ewing's sarcoma, myeloma	HT1080, RD-ES, AMO-1	0–15 μM	NA	Induction of ER stress and sensitization of bortezomib-resistant cells
[21]	Adult T-cell leukemia	MT-2, MT-4, C5/MJ SLB-1, HUT-102, MT-1, ED-40515	0–40 μM	30 mg/kg daily for 30 days, IP	Inhibition of NF- κB , inhibition of cell growth and induction of apoptosis
[22]	Cervical cancer	HeLa	0–50 μM	NA	Enhancement the anticancer activity of cisplatin

IP: intraperitoneal; OG: oral gavage; IR: ionizing radiation; NA: no data available.

In clinical oncology, such properties could be valuable in several contexts. The combination of RTV with radiotherapy may be particularly effective in radioresistant tumors such as head and neck and oral squamous cell carcinomas, where EMT and a robust DNA repair capacity often limit therapeutic efficacy [11,17]. Pairing RTV with cisplatin or other DNA-damaging chemotherapeutics may also enhance cytotoxicity, especially in tumors that have developed partial resistance [38]. Furthermore, combining RTV with PARP inhibitors could provide a synthetic lethality strategy, exploiting multiple vulnerabilities [39]. Finally, by promoting DNA damage and immunogenic cell death, RTV may increase the efficacy of immunotherapy when combined with radiation or genotoxic drugs [40,41].

6. Future Directions

Despite these promising findings, key steps are necessary before RTV can be clinically repositioned as an adjuvant and sensitizer for cancer treatment. Firstly, preclinical in vitro and in vivo works are needed to determine and define tumor selectivity versus normal tissue toxicity.

Indeed, tumor-specific vulnerabilities, including higher oxidative stress, reliance on or mutations in DNA repair and DNA damage response pathways, and altered metabolic and signaling networks [42], may render cancer cells more sensitive to the DNA-damaging effects of RTV at clinically relevant concentrations (10–20 μ M). Nevertheless, RTV concentrations at or above therapeutic levels can trigger apoptosis in normal hepatocytes, erythrocytes, enterocytes, and pancreatic cells [16,43–46], and induce oxidative stress in endothelial cells [47]. These harmful effects, however, can be attenuated by antioxidant compounds [48,49]. Despite this, the long record of RTV's safe clinical use supports a broad therapeutic window. Clinical studies in cancer patients have further confirmed its safety and antitumor activity when administered alone or with cytotoxic drugs [50–53], although some trials have reported less favorable results [54,55].

Overall, these findings highlight the need for further studies in additional normal and tumor contexts to better define the selectivity and safety of RTV.

In accordance, biomarkers such as γ -H2AX foci formation, known marker of DNA damage, or direct assays of ALKBH2 inhibition may help quantify its DNA-modulatory activity in vivo. Looking ahead, combinatorial strategies may further improve the therapeutic selectivity of RTV. Indeed, coupling RTV with precision genome-engineering tools such as base editors or prime editors, technologies that modify DNA without generating double-strand breaks, could help potentiate antitumor efficacy while reducing collateral genotoxicity in normal cells [56–58].

Importantly, clinical studies in radio- or chemo-resistant cancers should be planned to translate mechanistic insights into patient outcomes. To date, of a total of 1173 RTV clinical trials, 30 have focused on oncology. Notably, 17 of these trials have investigated RTV in combination with radiotherapy and/or genotoxic chemotherapy, including 9 completed (9 trials with RTV + genotoxic chemotherapy), 2 terminated (2 trials with RTV + radiotherapy + genotoxic chemotherapy), 2 withdrawn (2 trials with RTV + genotoxic chemotherapy), 1 with unknown status (1 trial with RTV + radiotherapy), and 3 currently recruiting (2 trials with RTV + genotoxic chemotherapy and 1 trial with RTV + radiotherapy + genotoxic chemotherapy) (Table 2). Available clinical data have primarily addressed safety and pharmacokinetic profiles, with no formal evaluation of DNA-damage or radiosensitization endpoints reported to date. Future investigations should therefore incorporate mechanistic biomarkers, such as γ -H2AX foci formation and ALKBH2 inhibition, to validate RTV's proposed activity on DNA damage response pathways in vivo.

Table 2. Summary of repurposed intervention of RTV in oncology clinical trials ¹.

NCT Number	Conditions	Interventions	Study Status	Phase	Response Rate
NCT03150368	Advanced Solid Tumors	ModraDoc006/r	Completed	Phase1	NA
NCT01173913	Cancer	ModraDoc001 10 mg capsules ModraDoc003 10 mg tablets and ModraDoc004 10/50 mg ModraDoc006 10 mg tablet	Completed	Phase1	PR 11.5% SD 23%
NCT03136640	Castration-resistant Prostate Cancer	ModraDoc006/r	Completed	Phase1	PR 10%
NCT03890744	Metastatic Breast Cancer Recurrent Breast Cancer	ModraDoc006/r	Completed	Phase2	NA

Table 2. Cont.

NCT Number	Conditions	Interventions	Study Status	Phase	Response Rate
NCT03383692	Neoplasm Metastasis	DS-8201 ^o Ritonavir Itraconazole	Completed	Phase1	CR 0% PR 10% SD 7% PD 0%
NCT02770378	Glioblastoma	Temozolomide Aprepitant Minocycline Disulfiram Celecoxib Sertraline Captopril Itraconazole Auranofin Ritonavir	Completed	Phase1 Phase2	SD 60% PD 40%
NCT04028388	Prostate Cancer Metastatic Castration-resistant Prostate Cancer	Docetaxel in Parenteral Dosage Form ModraDoc006/r	Completed	Phase2	CR 0% PR 44.1% SD 44.1% PD 11.8%
NCT00003008	Sarcoma	Indinavir sulfate Nelfinavir mesylate Paclitaxel Ritonavir Saquinavir mesylate	Completed	Phase2	NA
NCT03147378	Solid Tumor, Adult	ModraDoc006/r	Completed	Phase1	NA
NCT01124812	Non-Small Cell Lung Cancer	131I-L19SIP Radioimmunotherapy in Combination with External Beam Radiotherapy and Concurrent Chemotherapy	Terminated	Phase1	NA
NCT03066154	Prostatic Neoplasms	ModraDoc/r Androgen deprivation therapy Radiation therapy	Terminated	Phase1	NA
NCT05242926	Solid Tumor, Adult	ModraDoc006/r	Terminated	Phase1	NA
NCT05084456	Solid Tumor, Adult Impaired Liver Function	ModraDoc006/r	Withdrawn	Phase1	NA
NCT00637637	Cancer	Indinavir sulfate Ritonavir Radiation therapy	Unknown	Phase2	NA
NCT06710990	Advanced Breast Cancer	SHR-A1811 Ritonavir Itraconazole	Recruiting	Phase1	NA
NCT05150691	HER2-positive Advanced Solid Tumor	DB-1303/BNT323 Pertuzumab Injection Ritonavir Itraconazole	Recruiting	Phase1 Phase2	NA
NCT06428045	High-Grade Glioma	Abacavir Lamivudine Ritonavir Temozolomide Focal Radiotherapy	Recruiting	Phase1	NA

¹ From www.clinicaltrials.gov (accessed on 5 September 2025). CR: Complete Response; PR: Partial Response; SD: Stable Disease; PD: Progressive Disease; NA: no data available.

Conversely to novel experimental radio- or chemo-sensitizers, RTV has been in use for many years and its pharmacokinetics in humans are well known [59,60]: this would allow for a fast reuse of the drug in cancer patients.

7. Conclusions

RTV has long been used as an anti-HIV drug and a pharmacokinetic enhancer. Emerging data indicate that RTV also promotes DNA damage, thereby augmenting the efficacy of radio- and chemotherapy across multiple cancers: this provides a strong rationale for RTV clinical repositioning. Undoubtedly, the DNA-damaging effects of RTV could be harnessed in cancer therapy. Repurposing RTV as an adjuvant and sensitizer to genotoxic ra-

dio/chemotherapy could provide a promising avenue to overcome radio/chemo-resistance and improve cancer treatment outcomes.

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Abbreviations

The following abbreviations are used in this manuscript:

AKT	phosphoinositide 3 kinase/protein kinase B
CYP3A4	Cytochrome P450 3A4
DDR	DNA damage response
EMT	epithelial-to-mesenchymal transition
HIV	human immunodeficiency virus
ROS	reactive oxygen species
RTV	Ritonavir

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