Review

The potential role of sildenafil in cancer management through EPR augmentation

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Abstract: Enhanced permeation retention (EPR) was a significant milestone discovery by Maeda *et al.* paving the road for the emerging nanomedicine as a powerful tool in the fight against cancer. Sildenafil is a potent inhibitor of phosphodiesterase 5 (PDE-5) used for treatment of erectile dysfunction (ED) through the relaxation of smooth muscles and the modulation of vascular endothelial permeability. Overexpression of PDE-5 was reported in lung, colon, metastatic breast cancers and bladder squamous carcinoma. Accordingly, there has been a growing interest in using sildenafil as monotherapy or chemoadjuvant in EPR augmentation and management of different types of cancer. Sildenafil had been reported to increase the sensitivity of tumor cells of different origins to the cytotoxic effect of chemotherapeutic agents with augmented apoptosis mediated through inducing the expression of Bad and Bax proapoptotic proteins. It was also reported that the use of sildenafil prior to the administration of Doxorubicin (DOX) increased its EPR-related concentration in breast cancer tissues by 2 folds. Further, a substantial reason of anticancer chemotherapeutic failure is due to multidrug resistance (MDR), exacerbated by the overexpression of ATP-binding cassette (ABC) transporters such as ABCB1 and ABCCs. Sildenafil has demonstrated inhibitory effects on the efflux activity of ABCC4, ABCC5, ABCB1, and ABCG2, ultimately reversing MDR caused by these transporters. In this review, we critically examine the overall potential of sildenafil in enhancing EPR-based anticancer drug delivery pointing up the outcome of the most important related preclinical and clinical studies.

Keywords: Sildenafil; phosphodiesterase 5 inhibitors; drug repurposing; cancer; chemoadjuvant

1. Introduction

Sildenafil, (5-(2-ethoxy-5-((4-methylpiperazine-1-yl)sulfonyl)phenyl)-1-methyl-3-propyl-1H-pyrazolo[4,3]-d]pyrimidin-7(6H)-one) sold as citrate salt, is a drug primarily prescribed for the treatment of ED (Figure 1). Sildenafil exerts its biological effects through the inhibition of phosphodiesterase 5 (PDE-5) [1,2]. Phosphodiesterases are a class of enzymes responsible for the degradation of cyclic AMP (cAMP) or GMP (cGMP) to their respective nucleotides 5′-AMP and 5′-GMP.

Nowadays, eleven PDE isoforms have been identified [3]. These isozymes share an aminoacidic homology superior to 65% and differ for their tissue distribution and affinity toward cAMP or cGMP; the latter is specifically degraded by PDE-5, -6 and -9 [4–6]. PDEs

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exert their catalytic activity as homodimers [7,8]. In each monomer it is possible to highlight the presence of a zinc binding motif, a catalytic binding pocket, two allosteric sites able to bind cAMP or cGMP and a residue of serine in position 92 whose phosphorylation enhances the enzymatic activity through the activation of protein kinases A and G (PKA and PKG) [7,9]. PDEs regulate in an isoform-dependent manner different physiological roles such as platelet aggregation, inflammation, immune system activation, hormone secretion, vision, cardiac contractility and muscle metabolism, smooth muscle contractility, depression, calcium intracellular concentration, cell proliferation and penile erection [10]. The latter is an event that origins from the release of the gasotransmitter nitric oxide (NO) by nitrergic neurons and endothelial cells in case of sexual stimulation [11]. The physicochemical properties of NO allow it to diffuse into cells activating the enzyme soluble guanylyl cyclase (sGC) that in turn converts GTP into cGMP. In erectile tissues, cGMP triggers the phosphorylation of specific proteins involved in the modulation of the intracellular calcium ions concentration. A decreased concentration of calcium ions through the activation of Ca^{2+}-ATPase dependent transporters and BKCa channels produces the vasodilation of blood vessels in the corpus cavernosum, leading to a penile erection [12]. cGMP binding to the allosteric sites of PDE-5 facilitates the binding of additional cGMP molecules to the active site of the enzyme and the consequent abolishment of cGMP activity (Figure 1) [9].

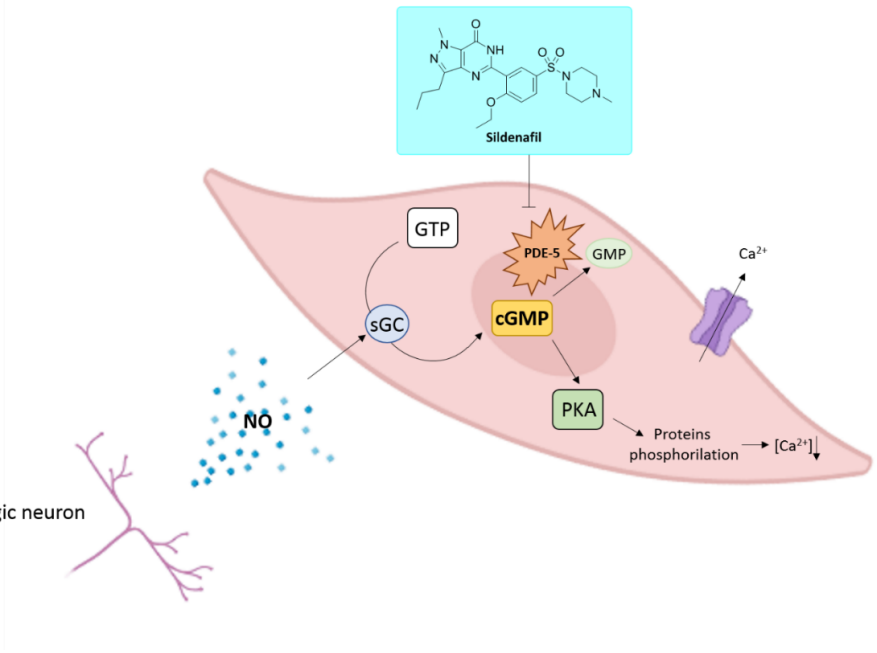

Figure 1. NO/sGC/cGMP pathway and sildenafil mechanism of action in erectile tissues and chemical structure of sildenafil.

After an oral administration, sildenafil exerts its biological properties in few minutes and its actions lasts around 12 hours. The drug is metabolized by hepatic enzymes and possesses inhibitory properties towards CYP3A4, altering the metabolism of other classes of drugs such as antimycotic azoles and HIV protease inhibitors [13,14]. Common side effects are represented by rhinitis, headache, flushing, cardiovascular effects and priapism. In addition, despite its selectivity towards the PDE-5 isozyme (IC50 = 3.5 nM), sildenafil possesses also the capability to bind PDE-6 (IC50 = 34 nM), an iso-form specifically expressed in rod and cone cells of the retina determining visual side effects [15,16].

Sildenafil is characterized by the presence of a pyrazo-lo[4,3]-d]pyrimidin-7(6H)-one nucleus that mimics the cGMP chemical structure. The pyrazole ring is decorated with alkyl substituents, whereas the pyrimidone ring is substituted with a phenyl ring bearing

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an ethoxy moiety and a N4-methylpiperazine-1-yl-sulfonyl moiety. Co-crystallization studies highlighted the binding mode of sildenafil to PDE-5 (Figure 3) [17]. The catalytic site of PDE-5 is characterized by the presence of four peculiar subsites. The M subsite (metal binding sub-site) possesses a zinc ion that takes interactions with histidine and aspartate aminoacidic residues and coordinates two water molecules. One aspartic residue and one water molecule coordinated by the zinc ion are also shared with a magnesium ion that takes interaction with four additional water molecules. The spatial disposition of the water molecules and the aminoacidic residues involved in the interaction with zinc and magnesium ions retained an octahedral geometry [18]. The second water molecule coordinated by zinc and unbonded to magnesium is involved in a hydrogen bond with an additional water molecule whose spatial disposition is assured by hydrogen bonds with Tyr612 and the unsubstituted nitrogen atom of the pyrazole ring of sildenafil. This specific hydrogen bond network seems to play a pivotal role in the inhibition of the PDE-5, indeed, it is speculated that this water molecule acts as the nucleophile responsible for the hydrolysis of the phosphodiester bond of cGMP [19]. The Q pocket (core pocket) accommodates the heterocyclic ring of sildenafil. In this subsite a Phe820 residue and the highly conserved Gln817 residue make a π-stacking interaction and a hydrogen bond with the amide function of the pyrimidinone ring, respectively. The hydrophobic subsite (H region) consists of a pocket in which highly lipophilic aminoacidic residues takes Van der Waals interactions with the ethoxyphenyl moiety linked to the heterocyclic core of sildenafil. Finally, a Tyr664 aminoacidic residue in the L region (lid pocket) undertakes a hydrogen bond with the N4 atom of the piperazine ring [18].

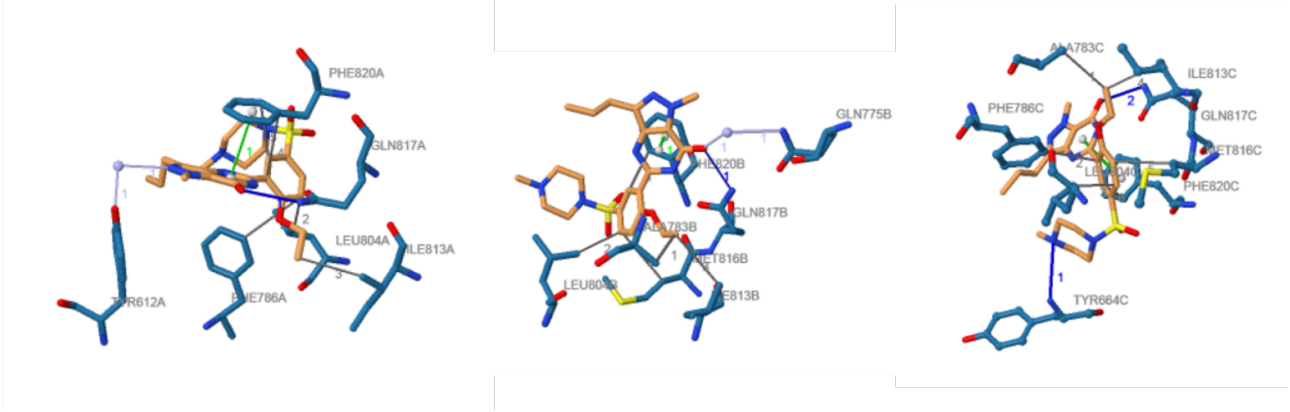

Figure 2. Figure 2. Docked position of sildenafil in the PDE-5 active site. Ligand is represented in orange; aminoacidic residues in blue. Hydrogen bonds are shown as solid blue lines, face-to-face stacking interaction in solid green lines, hydrogen bonds in dark solid grey lines, water bridges are represented in light solid grey lines. Image from the PLIP web service [20] using the PDB ID 2H42 [18]

The uncovering of sildenafil properties by Pfizer researchers represented one of the most resounding examples of serendipity in the drug discovery field (Figure 4) [11,21]. As a matter of fact, the cardiovascular research group operating in Pfizer in 1989 was looking for new drugs exploitable for the treatment of angina pectoris, a pathological condition caused by a temporary spasm of the coronary arteries with consequent reduced oxygen flow into the heart tissue [22]. The first clinical trials highlighted that UK-92,480 (sildenafil investigational code) did not possess any advantage when compared with other drugs commonly used for the treatment of angina pectoris, such as nitrates [23]. Indeed, doses of UK-92,480 administered intravenously or orally ranging from 20 to 200 mg weakly modified the hemodynamic parameters and potentiated the effects of nitrates. In response to these findings, UK-92,480 seemed to be not effective for the goal of the study and Pfizer researchers started to fear that the drug development of UK-92,480 could suffer a setback. Unexpectedly, among the limited number of side effects detected during these studies, penile erection resulted as the most surprising [24]. At the time of

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the research, ED was considered as a condition primarily originated by psychological disturbs and treated with invasive injections of vasodilating sub-stances in the penile tissues [11]. Moreover, PDE-5 was known to be principally localized in platelets and vascular smooth muscle cells, whereas its localization in the erectile tissues was never properly investigated. A subsequent study brought to light the presence of this specific enzymatic isoform in erectile tissues [25], allowing a better comprehension of the physiological processes that regulate penile erection [26]. In addition, this discovery confirmed that ED could be treated with orally administrable PDE-5 inhibitors because of the specific expression of this isozyme in erectile tissues, paving the way for the potential placing on the market of a class of compounds exploitable for an unmet clinical need. After twenty-one separate additional clinical trials carried out from 1993 to 1996 performed on a total number of about 3000 men aged 19 to 87 [11], the efficacy and patient's compliance of UK-92,480, later named as sildenafil, was definitely confirmed. These results determined UK-92,480 approval by the FDA on March 1998 in the United States and by the EMA on September 1998 [27] under the trade name of Viagra. The placing on the market of this drug represented a global market breakthrough for the treatment of ED, with more than 400 million U.S. dollars earned only in 1998 and more than 1 billion U.S. dollars.

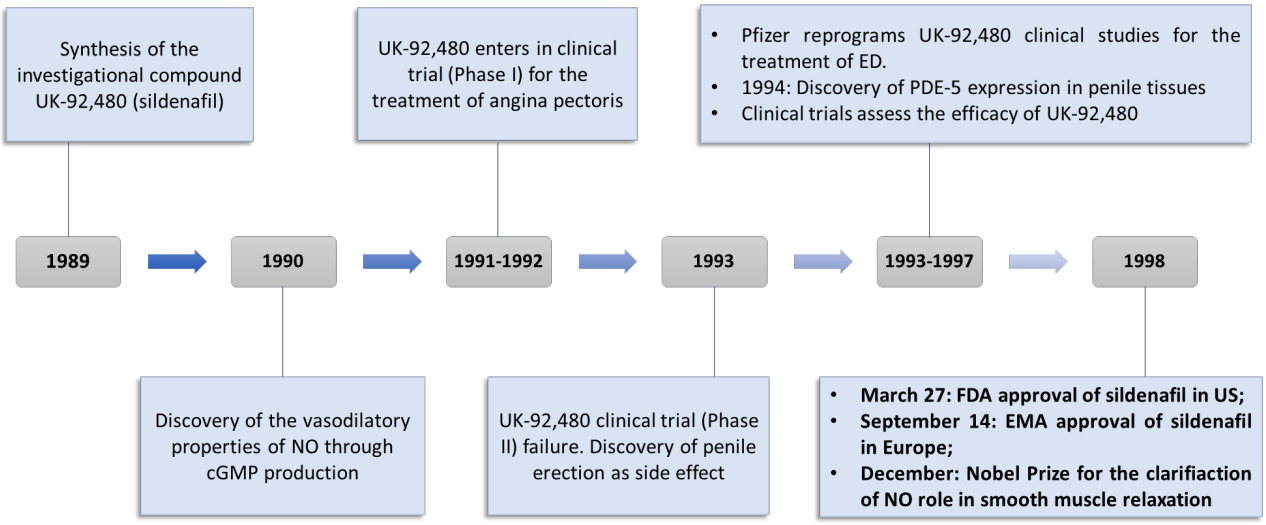

Figure 3. Timeline and milestones of sildenafil drug discovery.

In 2003 additional PDE-5 inhibitors have entered the market (vardenafil and tadalafil), whereas in recent years avanafil, mirodenafil, lodenafil and udenafil have been approved in a limited number of countries (Figure 5) [28].

In 2010, sildenafil's patent has expired, and several industries started the production of this drug under generic names. Nevertheless, several clinical trials have been carried out in order to assess the efficacy of sildenafil for the treatment of other disabling pathologies [29–32].

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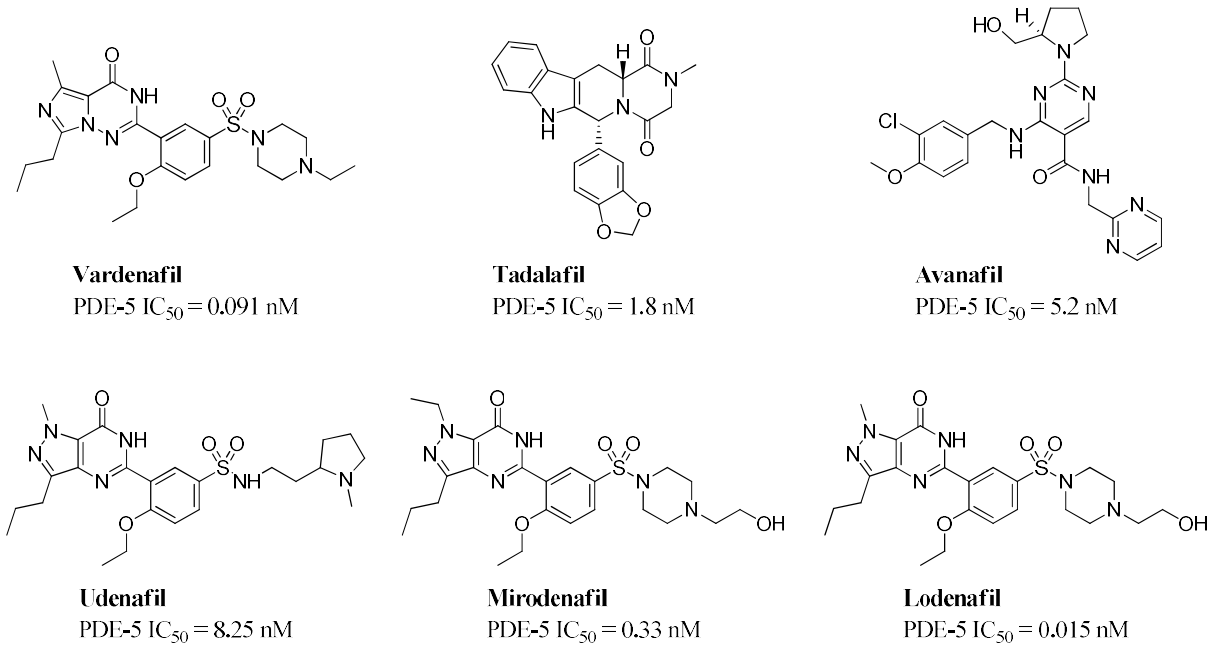

Figure 4. Chemical structures and IC_{50} values of commercial PDE-5 inhibitor.

2. Drug repurposing approach for the identification of new therapeutic application

In spite of increased understanding of prevention, diagnosis, therapy, and prognosis of human maladies, translation of this whole set of knowledge into new drugs has been far slower than estimated. A new drug-discovery project generally starts when there is an unmet clinical need that is the primary driving motivation. Initial efforts often occur in academia that produces data to support a hypothesis that may result in the identification of a new target or a new therapeutic approach in a specific disease. In our time, however, drug discovery and development processes are resource and time intensive and highly multifaceted requiring multi-disciplinary profiles and innovative approaches. Attrition rate is another relevant aspect that the global pharmaceutical industry has to take in serious account when approaching a new discovery project. Latest estimations suggest that it takes more than 10 years and around 2 billion U.S. dollars for a new drug to reach the market. There is a growing pressure to set up cheaper and more effective ways to bring safe and efficacious drugs to the market. Within this framework, the drug discovery process is unceasingly experiencing changes and adjustments to achieve improvements in efficiency, productivity, and profitability. In this context, the so-called drug repositioning (or repurposing) process is attracting growing interest [33]. This strategy implies the identification of new therapeutic applications different form the original regulatory indication for approved or investigational drugs. Benefits of this strategy includes tremendous savings of time and money, low risk of failure since the majority of preclinical and clinical trials, safety assessment and, sometimes, pharmaceutical formulation have been completed. Finally, yet importantly, re-purposed drugs may highlight novel targets and pathways that can be further investigated. In the past, the most significant examples of drug repurposing have been mainly based on serendipity rather than on a systematic approach. Once an off-target or a new on-target effect was detected, it was the object of further investigation and/or commercial exploitation. An outstanding example is represented by Zidovudine, originally developed as anticancer agent, become the first FDA-approved drug for the treatment of HIV and was identified from an in vitro screening of compound libraries [34]. Other remarkable examples include thalidomide originally developed for morning sickness and then, on the basis of pharmacological analysis, has been approved for the treatment of erythema nodosum leprosum and multiple myeloma [35]. Minoxidil, originally indicated for the treatment of hypertension, was

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discovered by means of a retrospective clinical analysis. However, sildenafil represents maybe the foremost example. Originally investigated for angina, represents maybe a perfect example of retrospective clinical analysis. Sildenafil, when repurposed by Pfizer the first time in the late nineties for the management of ED, it held a market-leading 47% share of ED drug market in 2012, with worldwide sales calculation around 2 billion U.S. dollar [36]. Soon after its approval as Viagra, the discover of the upregulation of PDE5 gene expression in pulmonary hypertensive lungs boosted further preclinical and clinical studies on sildenafil to test the role of PDE5 selective inhibitors in lung diseases [37]. Later in 2005, the drug has been repurposed once more for the treatment of pulmonary arterial hypertension and approved under the trade name Revatio [12,38]. Recently, other indications for which sildenafil has been studied include Raynaud's disease, digital ulcer, hearth failure, hypertensive cardiac hypertrophy, cerebral circulation and different types of cancers including lung and colorectal malignancies [39,40].

3. In vitro and in vivo applications of sildenafil in cancer treatment

Many studies reported the use of sildenafil in combination with chemotherapeutic agents in treatment of a variety of cancers. Das, A. *et al.* reported an increase in chemotherapeutic efficacy of DOX when co-administered with sildenafil in vitro on PC-3 and DU145 human prostate cancer cells. It was shown that combination therapy resulted in a relatively higher apoptotic rate on tumor cells by enhancing reactive oxygen species generation, reducing B-cell lymphoma-extra large (Bcl-xL) expression, phosphorylating BAD and up-regulating caspase-3 and caspase-9 activities [41]. Further investigations on the molecular mechanisms involved in sensitization of prostate cancer cells by sildenafil outlined the role of CD95 in DOX-mediated apoptosis [42]. The effect of sildenafil in enhancing the anticancer properties of DOX was eliminated when CD95 apoptosis-inducing death receptor was knocked down using siRNA. However, this was not the case when cells were treated with DOX alone. In addition, the combination therapy induced downregulation of Fas associated phosphatase-1 (FAP-1) expression, a known inhibitor of CD95-mediated apoptosis, increasing cellular death and reducing tumor viability. Moreover, cells co-treated with sildenafil and DOX showed a reduced expression of both long and short forms of caspase-8 regulating enzymes Fas-associated death domain (FADD) interleukin-1-converting enzyme (FLICE)-like inhibitory protein (FLIP-L and -S) which are involved in the regulation of cellular apoptosis relative to DOX-monotherapy [42]. Comparable results were reported for using the same therapeutic combination in treatment of 4T1 murine breast cancer cells where synergistic activity was observed [43]. In vitro studies examining the potentiation of the antitumor activity of cisplatin when given in conjugation with sildenafil on MCF-7 human breast cancer cells showed a dose-dependent cytotoxic effect of sildenafil illustrating its potentiation effect on the chemotherapeutic agent [44]. Similar results were obtained upon co-treatment of MCF-7 and MDA-MB-468 human breast cancer cells with cisplatin and sildenafil which was accompanied by a significant increase in accumulation of reactive oxygen species (ROS) into the extracellular environment in both breast adenocarcinomas cell lines [45].

The effect of co-administration of vincristine and sildenafil on PC-3 and DU145 human prostate cancer cell lines showed that a significant increase in vincristine-induced mitotic arrest and mitotic index [46]. The probability of cells being held in metaphase were dramatically increased in presence of sildenafil. This was particularly relevant in the tripolar spindle and multiple spindle poles. Nevertheless, a non-significant decrease in the level of cytokinesis was observed when cells responsive to vincristine were treated with sildenafil. Interestingly, the phosphorylation of Bcl-2 with caspase activation amplification including caspase-3, -8, and -9, and cleavage of poly [ADP-ribose] polymerase 1 (PARP-1), a caspase-3 substrate, was markedly increased when sildenafil was co-administered with vincristine. Additionally, sildenafil was shown to enhance vincristine-induced perturbation of microtubule–kinetochore interactions incurring higher apoptotic effects [46].

Roberts, J. L. *et al*. reported that combination therapy of curcumin and sildenafil may induce gastrointestinal tumor cell death in HCT116, HT29, HuH7, HEP3B and HEPG2 human gastrointestinal tumor cells through endoplasmic reticulum stress, reactive oxygen/nitrogen species and increasing autophagosome and autolysosome levels prompting cancer cellular death [47]. Similar results were obtained when studying the effect of co-administration of curcumin and sildenafil on immunocompetent BALB/c mice implanted with CT26 murine colorectal cancer cells where the use of sildenafil and curcumin as chemoadjuvants has significantly increased efficacy and enhanced the cytotoxic effect of 5-flurouracil and anti-PD1 immunotherapy in vivo [48].

The therapeutic efficacy of docetaxel and sildenafil in advanced prostate cancer was investigated by stimulating nitric oxide - cyclic guanosine-3′,5′-monophosphate (NO-cGMP) signaling. Human prostatic cancer (C4-2B) cells revealed an over expression of functional phosphodiesterase type 5 (PDE5) and its role with NO for aberrant cGMP accumulation. It was suggested that a sub-therapeutic dose of docetaxel and a physiologically achievable sildenafil concentration could induce synergistic activity by increasing cGMP and blocking cells at G0/G1; inhibiting cell growth and inducing apoptosis. Similar results were observed in syngeneic cell lines and Pten cKO derived tumoroids where an increase in caspase-3 and PARP cleavage was detected [49]. The combination treatment demonstrated a significant decrease in tumoroid size and growth, with loss of integrity, apoptosis, condensed structure and structural blebbing [50].

The cytotoxicity of sildenafil/crizotinib loaded poly(ethylene glycol)-poly(DL-lactic acid) (PEG-PLA) polymeric micelles on MCF-7 human breast cancer cell lines was studied. Micelles with an average size between 93 and 127 nm and an encapsulation efficiency percentage (EE%) of both medications (>70%) were prepared using the solvent displacement method. In vitro cytotoxicity assays using crizotinib alone displayed 22% cellular viability relative to 10% only upon co-administration of sildenafil, i.e. 2.2 fold decrease in cell viability, after treatment for 48hrs. This was attributed to previous reports on the wide inhibitory effect of sildenafil on several ATP-binding cassette (ABC) efflux transporters, henceforth overcoming cancer cell resistance and promoting their apoptosis [51]. Co-delivery of these medications using nanoparticles further decreased the cell viability to 4% illustrating the potential impact of formulation designs on enhancing the therapeutic outcomes of this regimen [52].

In a different study, nanostructured lipid carrier (NLCs) co-loaded with DOX and sildenafil citrate and tagged with arginyl-glycyl-aspartic acid (RGD) were prepared and their effect on human lung carcinoma A549 cells was studied [53]. The drug-loaded NLCs were prepared by homogenization method producing an optimum formula having an average size, polydispersity index, zeta potential and EE% for DOX and sildenafil of 80.5 nm, 0.23, -18.5, 56.04 ± 1.25% and 81.62 ± 3.14%, respectively. The use of co-loaded NLCs induced higher cytotoxicity and cancer cell apoptosis relative to the free drug. It was suggested that this may be due to the enhanced cellular uptake and accumulation of drugs associated with integrin mediated endocytosis and ABC transporter inhibition. Real-time PCR also revealed that sildenafil reduced the expression of ABCC1 and nuclear factor erythroid 2 related factor 2 (Nrf2) proteins which incurred an increased intracellular concentration of anticancer drugs as previously reported [53,54].

In vivo studies using athymic male BALB/cAnNCr-nu/nu mice bearing prostatic cancer showed that the coadministration of sildenafil increased the efficacy of DOX whilst reducing DOX-associated cardiac dysfunction [41]. Immunohistochemistry demonstrated that the active form of caspase-3 was induced in tumors from sildenafil- and DOX-treated mice compared with DOX-treated or nontreated control groups, henceforth explaining the relatively higher tumor volume reduction with the co-treatment. Furthermore, doppler echocardiography showed a marked improvement in the left ventricular fractional shortening (LVFS) and left ventricular ejection fraction (LVEF) with sildenafil-DOX co-treatment rather than DOX alone. These results suggest a relatively lower systemic cytotoxicity associated with the co-treatment relative to monotherapy [41].

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Treatment of female Balb/c mice inoculated with 4T1 murine mammary carcinoma cells with sildenafil/DOX combination therapy also demonstrated a significant reduction of tumor growth [43]. It was suggested that this effect is due to a higher migration of effective immune cells to the tumor site due to the vasodilatory effects of sildenafil, rather than an inherent cytotoxic effect of the drug. The results were in correlation with in vitro studies which demonstrated lack of anticancer properties of sildenafil. Animals treated with sildenafil-DOX combination showed a 4.7 reduction in tumor size with a 2.7-fold increase in drug concentrations in comparison to DOX alone. Interestingly, when DOX was loaded into styrene maleic acid (SMA) micelles and administered to the mice after sildenafil treatment, it showed a statistically insignificant increase in tumor accumulation relative to SMA-DOX alone. This was not the case when dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) was loaded in SMA micelles and co-delivered with sildenafil, where a statistically significant 3-fold increase was observed relative to SMA-DiI alone [43].

Similarly, other in vivo studies using combination of sildenafil and cisplatin showed a significant decrease in tumor volume in mice bearing breast cancer tumor relative to the control group. Investigation of the local tissue microenvironment, apoptosis, and proliferation of the tumor cells after treatment with combination therapy showed an increase in caspase-3 levels with a considerable decrease in tumor necrosis factor-α contents, angiogenin, and vascular endothelial growth factor expression. However, the expression of Ki-67 nuclear protein which is usually present during the late G1, S, G2 and M phases of the cell cycle failed to show any significant changes when compared to the control group [44].

Muniyan, S. *et al.* orthotopically implanted luciferase-labelled C4-2Bcells into the dorsolateral lobe of the prostate in immunodeficient mice to investigate the therapeutic efficacy of co-administration of docetaxel and sildenafil in advanced prostate cancer [50]. The therapeutic combination significantly lowered tumor weight compared to docetaxel alone. Further exploration in the molecular pathways responsible for this phenomenon identified a lower percentage of Ki67-positive nuclei and a higher frequency of cleaved caspase-3 positive cells relative to groups treated with monotherapy, thus promoting apoptosis and tumor regression [50]. Likewise, Hsu, J.-L. *et al.* reported the synergestic effects between vincristine and sildenafil in PC-3-derived cancer xenografts in nude mice demonstrating a decrease in tumor weight relative to the single chemotherapeutic agent [46].

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Table 1. Examples of in vitro and in vivo studies for the effect of sildenafil in different types of cancer

Cancer	Type of study	Tumor Model	Therapy	Therapeutic outcome	Ref.
Prostate cancer	In vitro	PC-3 and DU145 prostate cancer cells	Sildenafil (10 μM)	No significant changes in % Cell death relative to control	[41]
			DOX (1.5 μM with PC-3 and 0.5 μM with DU145)	7.52% and 45.01% cell death in PC-3 and DU145 cells, respectively.	
			DOX (1.5 μM with PC-3 and 0.5 μM with DU145) + Sildenafil (10 μM)	18.71% and 56.82% cell death in PC-3 and DU145 cells, respectively.	
	In vivo	Athymic male BALB/cAnNCr-nu/nu mice injected with PC-3 cells and 50-μL matrigel matrices	DOX (1.5 mg/kg)	Tumor weight/Body weight ratio = 0.015	
			Intraperitoneal DOX (1.5 mg/kg) + Sildenafil (5 mg/kg) OR intraperitoneal DOX (3 mg/kg) + oral Sildenafil (10 mg/kg)	Tumor weight/Body weight ratio = 0.010	
Breast cancer	In vitro	4T1 mammary carcinoma cells	DOX (1μM)	50% cell death	[43]
			Sildenafil (10,30,100μM)	No significant changes relative to control	
			DOX (1μM) + Sildenafil (1μM)	72.2% cell death	
			DOX (1μM) + Sildenafil (30μM)	91.9% cell death	
			DOX (1μM) + Sildenafil (100μM)	97.6% cell death	
	In vivo	Female Balb/c mice injected with 4T1 mammary carcinoma cells	DOX (5 mg/kg)	Tumor volume = 570%	
			Sildenafil (1 mg/kg)	Tumor volume = 400%	
			DOX (5 mg/kg) + Sildenafil (1 mg/kg)	Tumor volume = 121.3%	
Breast cancer	In vitro	MCF-7 breast cancer cells	Sildenafil	IC_{50} =14 μg/mL	[44]
			Cisplatin	IC_{50} = 4.43 μg/mL	
			Sildenafil + Cisplatin	IC_{50} = 3.98 μg/mL	
	In vivo	Swiss albino female mice injected with Ehrlich ascites carcinoma (EAC) cells	Sildenafil (5 mg/kg)	30.4% decrease in tumor volume	
			Cisplatin (7.5 mg/kg)	58.8% decrease in tumor volume	
			Sildenafil (5 mg/kg) + Cisplatin (7.5 mg/kg)	79% decrease in tumor volume	
Colorectal Cancer	In vitro	HT-29, SW480, SW620, HCT116 and SW1116	Sildenafil	IC_{50} (72hrs) = 190.91 μM in HT-29	[55]

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		colorectal cancer cells		217.27 μM SW480 206.68 μM SW620 246.20 μM HCT116 271.22 μM SW1116	
	In vivo	Balb/c nude mice injected with SW480 or HCT116 colorectal cancer cells	Sildenafil (50 mg/kg) and (150 mg/kg)	In SW480, 40.1% and 57.8% tumor inhibition with 50 mg/kg and 150 mg/kg, respectively. In HCT116, 13.3% and 61.4% tumor inhibition with 50 mg/kg and 150 mg/kg, respectively.	
Prostate Cancer	In vivo	Nude mice were injected with PC-3 prostate cancer cells	Sildenafil (10 mg/kg) Vincristine (0.5 mg/kg) Sildenafil (10 mg/kg) + Vincristine (0.5 mg/kg)	Tumor weight = 969.9 ± 92.2 mg Tumor weight = 623.5 ± 132.2 mg Tumor weight = 207.6 ± 36.7 mg	[46]
Breast Cancer	In vitro	MCF-7 Breast cancer cells	Sildenafil Crizotinib Crizotinib + Sildenafil Blank PEG-PLA micelles Crizotinib loaded PEG-PLA micelles Crizotinib (55.25 μM) /Sildenafil (40.33 μM)-coloaded PEG-PLA micelles	No significant changes in % cell viability relative to control IC_{50} = 34.19 and 22% cell viability IC_{50} = 3.34 and 10% cell viability No significant changes in % cell viability relative to control 14% cell viability 4% cell viability	[52]
Lung cancer	In vitro	A549 human lung carcinoma cells	DOX DOX + Sildenafil DOX /Sildenafil-coloaded NLC DOX /Sildenafil-coloaded NLC-RGD	29.87 % cell death 34.69 % cell death 38.37 % cell death 44.32 % cell death	[53]

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4. The role of sildenafil in circumventing anticancer drug resistance

MDR is a complex process in which cancer cells evolves to evade the deleterious effects of anticancer chemotherapy. A plethora of biological strategies had been described in association with the development of MDR. Enhanced drug metabolism, gene amplifications, increase in DNA damage repair, epigenetic regulation of the drug targets, and autophagy all have been described.

Among different process of drug resistance overexpression of active transporters that actively efflux substrates of different chemical/ biological natures, is the most studied pathway, notably the increase of drug efflux pumps ATP-binding cassette (ABC) transporters [56–60]. ABC transporter comprises ABCCs (multidrug resistance-associated proteins (MRP)), ABCB1 (P-glycoprotein/MDR1), and ABCG2 (BCRP/MXR/ABCP) all were reported to be overexpressed in cancer developing the MDR. This superfamily transporter system are mainly integral membrane proteins. These proteins convert the energy that comes from ATP hydrolysis into the translocation of substrates across the membrane's bilayer either into the cytoplasm or out of the cytoplasm. This movement is facilitated by a pair of transmembrane domains (TMDs). When transporters were overexpressed in cancer cells, they pump out the intracellular drugs, therefore decreasing the drug's effect that make them contributing to cell drug resistance [61]. cGMP was implicated as substrate for ATP-binding cassette (ABC) transporters in the Multidrug resistance (MDR) cancer cells [59,60]. Subsequently, sildenafil was investigated as potential player for reversing MDR in cancer cells.

Sildenafil increased the level of second messenger's cGMP through inhibiting PDE5 which is considered to be substrates for ABCC4/ human MDR protein 4 (MRP4) and ABCC5/ human multidrug resistance protein5 (MRP5) that led to inhibits the efflux pump activity. Furthermore, inhibiting the activity of ABC transporters such as ABCB1 and ABCG2 thereby increasing MDR cell's sensitivity to various drugs. Moreover, the suppression of PDE5 could activate the cGMP-PKG pathway mediates many processes causing cellular apoptosis or growth suppression (cell cycle arrest) of cancer cells [62].

Shi et al. demonstrated the effect of sildenafil on ABC transporters using ABC-mediated MDR on cancer cells. The cytotoxicity assays and drug accumulation results demonstrate that sildenafil remarkably sensitized the ABCB1-overexpressing cells to the ABCB1 substrates (colchicine, vinblastine, and paclitaxel) with a high accumulation rate of the paclitaxel inside the cells. Similar effect on ABCG2-overexpressing cells was noted in relation to the substrates (flavopiridol, mitoxantrone, and SN-38) with significant accumulation of mitoxantrone in contrast sildenafil, had no effect on ABCC1-overexpressing cells and its substrate tested (vincristine). All together, these data strongly suggest a potential role for sildenafil in reversing anticancer drug resistance [63].

5 . Sildenafil and anticancer drug delivery through EPR augmentation

PDE5 inhibitors such as sildenafil had demonstrated its effect on smooth muscle layers of blood vessels leading to vasodilation in tissues that express the specific isoenzyme. Indeed , one known side effect of this class of drugs is systemic hypotension that denotes the susceptibility of normal vascular cell types to PDE5 inhibitors [64].

Smooth muscle relaxation thereby modulates vascular endothelial permeability that increases the inflow of blood to Improving the blood flow in the normal and pathological tissues as inflamed tissues and tumor tissues leading to the accumulation of nanoparticles of molecular weight exceeding 40kD and augmenting preferential drug targeting in the diseased tissues like tumors. This accumulation normally occurs due to the abnormalities in tumor vascular include of poorly aligned and faulty vascular endothelial cells that have wide fenestrations of up to 4 μm [65–67]. Traditionally, EPR effect involves two aspects. First, the drug preferential biodistribution that is related to the size of the drug and the delivery vehicle applied to achieve the differential accumulation of the drug in tumor tissues. As the size of the drug and the delivery vehicle is more than the limit of

renal excretion threshold, nanoparticles usually exhibit increased plasma half-life. Second, the EPR effect involves retention of the nano-based system due to lack of efficient lymphatic clearance [68–70].

Unfortunately, a very slim volume of existing literature examines the response of tumor vasculature to PDE5 inhibitors. PDE inhibition could potentially result in improvement of blood supply to the tumor tissues through similar mechanisms employed for ED. Greish et al. demonstrated that using sildenafil in conjunction with DOX, increased the concentration of the anticancer drug in tumor tissues by 2.7 folds, and eventually resulted in 4.7 folds improved anticancer activity against the 4T1 breast cancer in mice. This work suggests a positive effect of PDE5 inhibitors to further augment enhanced permeability and retention (EPR) effect on EPR effect [43]. A relevant study by Black et al., demonstrated the effect of PDE5 inhibitors on enhancing tumor vascular permeability in the brain tumor model of 9L gliosarcoma-bearing in rats. Sildenafil administration increased the tumor capillary permeability in comparison to the normal brain capillaries that showed no significant increase in vascular permeability. Additionally, the study proved a synergistic effect of the use of anthracycline chemotherapy combined with the sildenafil and further improved the survival by nearly 2 folds longer than the group treated with the chemotherapeutic agent alone [71]. Another work by Zhang et al. provided a further direct evidence of the in vivo on the potential of PDE 5 inhibitors on augmenting EPR mediated anticancer chemotherapy. In their study the team employed a combined micelle incorporating both cisplatin and sildenafil. The team proposed that tumor acidity can preferentially release the PDE5 inhibitor from the micelle, further augmenting its concentration in tumor tissues. This strategy was proved effective in increasing both drug accumulation and anticancer activity in the tested cancer model of B16F10 melanoma in C57BL/6 mice. All together indicating a potential and promising rule for PDE5 inhibitors in augmenting EPR based anticancer drug delivery [72].

6. Clinical studies

The use of sildenafil in management of different types of cancer has been the subject of various clinical trials (http://www.clinicaltrials.gov) (Table 2). A number of clinical trials such as NCT00142506, NCT00544076, NCT00057759 and NCT00511498, evaluated the use of sildenafil alone or in combination with alprostadil or hyperbaric oxygen therapy in management of ED. Those trials focused on restoring the erectile function for patients with prostate cancer after radiotherapy or nerve-sparing prostatectomy. Clinical trial NCT02106871 was designed to assess the use of sildenafil monotherapy in treatment of fatigue in patents with pancreatic cancer. It is suggested that sildenafil increases protein synthesis, alters protein expression and nitrosylation, and reduces fatigue in human skeletal muscle especially in patients with reduced skeletal muscle functions [73]. The concept has yet to be clinically tested as the study was terminated due to lack of funds. The ability of sildenafil monotherapy to improve renal functions in patients with kidney cancer after partial nephrectomy and protect the kidney from the side effects of surgery was investigated in clinical trial NCT01950923. The study involved the oral administration of sildenafil to 30 patients prior to surgery followed by assessment of kidney functions. The trial was completed but the results has yet to be reported. In clinical trial NCT00165295, sildenafil has been tested in treatment of Waldenstrom's Macroglobulinemia (WM) a rare and incurable type of non-Hodgkin lymphoma. It was suggested that sildenafil blocks the function of several proteins necessary to the survival of cancer cells and laboratory tests have shown that it can destroy WM cells [74]. The study involved 30 patients who received incremental doses of sildenafil orally for 2 years. The clinical trial has been completed with no reported side effects, but the complete results of the study are not published yet. Sildenafil was also tested for the treatment of Lymphangioma in paediatric patient in clinical trial NCT01290484. The results showed a significant decrease of lymphatic malformation in 4 out of 7 patients included in the study

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after oral administration of sildenafil for 20 weeks with no observed complications in any subject [75].

The use of sildenafil as a chemoadjuvant in treatment of different types of cancers was investigated. The clinical trial NCT01375699 investigated the use of sildenafil as a cardioprotective agent in female patients primarily with breast cancer treated with DOX against the cardiotoxic effects of the drug. Patients were given oral sildenafil daily for one week prior to the scheduled first dose of DOX. The treatment continued until 2 weeks after last scheduled dose of DOX and multiple biomarkers for cardiotoxicity were measured [76]. The results showed that adding sildenafil to DOX chemotherapy is safe and well tolerated but did not significantly improve cardiac protection during chemotherapy when compared to control group. The trial NCT00752115 used sildenafil combination with chemotherapeutic agents such as carboplatin and paclitaxel in patients with advanced non-small cell lung cancer to improve the biodistribution and efficacy of the chemotherapeutic agents. Patients received a weekly dose of 50 mg sildenafil orally and progression free survival was monitored. The phase I clinical trial NCT02466802 assessed the use of regorafenib in combination with sildenafil in patients with progressive advanced solid tumors. The study results showed that the drug combination is safe, and that the lethality of this combination could be enhanced in vitro and in vivo by the addition of neratinib to the treatment regimen in a colorectal cancer model. Accordingly, it was further recommended to perform a phase I trial in colorectal cancer patients using the combination of the three drugs [77]. The phase II clinical study NCT01817751 is currently investigating the use of sildenafil as chemoadjuvant in treatment of patients with recurrent high-grade glioma. Orally administered sildenafil twice a day for four weeks is used in combination with sorafenib and valproic acid to test its ability to increase the concentration of the chemotherapeutic agents in the brain and preventing the growth of tumor cells by blocking BCG2 drug efflux pump in the blood brain barrier.

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Table 2. Examples of clinical trials using sildenafil in treatment of different types of cancers*

Types of cancer	Treatment	Objective	Stage
Pancreatic cancer	Sildenafil	Management of fatigue in cancer patient undergoing chemotherapy	Phase I
Non-small Cell Lung Cancer	Sildenafil, Paclitaxel, Carboplatin	Improvement in distribution and efficacy of cytotoxic anticancer agents	Phase II, III
Prostate cancer	Sildenafil	Management of ED during and after radiotherapy with or without hormone Therapy	Phase III
	Sildenafil, Alprostadil	Management of ED post-operatively in patients undergoing nerve-sparing robotic-assisted radical prostatectomy	Phase III
	Sildenafil	Investigate the effect of dosage regimen on ED in patients after nerve-sparing laparoscopic radical prostatectomy	Not applicable
	Sildenafil, Hyperbaric oxygen therapy	Management of ED in patients after nerve-sparing radical retropubic prostatectomy	Phase IV
Solid Tumor	Regorafenib Sildenafil	Investigation of the antitumor effects of the regorafenib and sildenafil combination, the pre-treatment expression of phosphodiesterase type 5 (PDE5) in tumor samples and the impact of sildenafil on the pharmacokinetics of regorafenib	Phase I
Kidney Tumor	Sildenafil	Improving Postoperative Kidney Function in Patients With Kidney Cancer undergoing Robotic Partial Nephrectomy	Phase I
Colorectal cancer	Sildenafil Vacuum erection device (VED)	Management of ED After Laparoscopic Resection	Phase IV
Breast cancer	Sildenafil Doxorubicin	Improving anti-tumor effects of DOX and protection from cardiac toxicity	Phase I
Brain cancer and glioblastoma	Sildenafil Sorafenib Tosylate Valproic Acid	Increase the concentration of anticancer drug in the brain and stop the growth of tumor cells by blocking BCG2 drug efflux pump in the blood brain barrier	Phase II
Waldenstrom Macroglobulinemia	Sildenafil	Treatment by blocking the function of several proteins necessary to the survival of cancer cells	Phase II

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Myelodysplastic syndrome (MDS)	Nivolumab Cytarabine Sildenafil	Studying the pathogenesis and resistance of myelodysplastic syndrome using combination therapy	Phase I, II

*Source: https://clinicaltrials.gov/

5. Conclusion and future recommendations

The paradigm of drug repurposing remains of significant interest for the pharmaceutical and health care communities. Deeper understanding of the molecular pathology and pharmacology of the current therapeutic entities in the market plays an important role in the utilization of current resources in the management of various diseases. Further to Meade's visionary discovery of EPR, he recommended further augmentation of this key biological effect by manipulating vascular dynamics at macro, and micro-organizational levels. Sildenafil has demonstrated its ability in enhancing anti-cancer drug delivery through the EPR effect, prompting significant elevation of intratumoral drug concentrations and subsequent cellular death. In addition, sildenafil has demonstrated its implication in the modulation and potentiation of chemotherapeutic agents in a range of different types of cancer. This has been outlined in several in vitro and in vivo studies through the downregulation of Bcl-xL and FAP-1 expression, enhancing ROS generation, phosphorylating BAD and Bcl-2, up-regulating caspase-3,8,9 activities, blocking cells at G0/G1 cell cycle phase, overcoming cancer cell resistance by inhibiting several ABC transporters through cGMP elevation, and increasing autophagosome and autolysosome levels; inducing tumor cell death.

Despite several clinical studies being underway, the need for further use on patients remains of paramount importance to further understand the clinical impact they may perceive. These studies could possibly include the application of novel drug delivery formulations for combination therapies such as passively and actively targeting nanoparticles, external stimuli responsive systems using light, focused ultrasound and magnetic fields to release the drug therapy at the desired site of action, and controlled release formulations where sildenafil may precede the chemotherapeutic agent; inducing its chemosensitizing action first and promoting higher cytotoxicity action of the latter. Such systems could certainly increase the efficacy and safety profiles of current oncological agents, enhancing the patient's quality of life and achieving a definite therapeutic outcome.

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