

Mini-review

The redox-active nanomaterial toolbox for cancer therapy

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ABSTRACT

Advances in nanomaterials science contributed in recent years to develop new devices and systems in the micro and nanoscale for improving the diagnosis and treatment of cancer. Substantial evidences associate cancer cells and tumor microenvironment with reactive oxygen species (ROS), while conventional cancer treatments and particularly radiotherapy, are often mediated by ROS increase. However, the poor selectivity and the toxicity of these therapies encourage researchers to focus efforts in order to enhance delivery and to decrease side effects. Thus, the development of redox-active nanomaterials is an interesting approach to improve selectivity and outcome of cancer treatments. Herein, we describe an overview of recent advances in redox nanomaterials in the context of current and emerging strategies for cancer therapy based on ROS modulation.

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Introduction

Reactive oxygen species (ROS) are capable of exerting different effects according to their nature, localization and levels [1]. An imbalance on ROS production and scavenging can lead to a sustained increase of ROS levels and to a pro-oxidant state that have been associated with a broad spectrum of pathological disorders, including cancer. ROS are involved in all aspects of carcinogenesis [2], as well as in non-surgical cancer treatments [3]. ROS modulation induced by conventional radio- and chemotherapy may impact on tumor outcome, although with poor selectivity and high toxicity. Thus, novel therapies are pursued in order to solve these issues. Therefore, studies on cancer treatments based on ROS modulation have grown in the last decade and, in recent years, research on nanomaterials for medical applications contributed to generate novel nanostructures

with redox-active properties that may improve oncologic diagnosis and therapies.

Approaches for cancer treatment based on ROS modulation

ROS are known to have a double-edged sword property in determining cell fate, being able to increase or decrease the risk of cancer based on diverse conditions [4, 5]. Considering this, both pro- and antioxidant approaches were developed or are in development (Fig. 1 and Table 1), although nowadays, they are not clinically relevant yet. Redox alterations in cancer cells are very complex and the simple addition of ROS-generating or depleting agents may not always lead to a preferential killing of cancer cells [6, 7]. Thus, understanding the complex redox system in cancer cells is critical to achieve an adequate therapy. Under physiological conditions, normal cells maintain redox homeostasis with a low level of basal ROS by controlling the balance between ROS generation and elimination. Their reserve antioxidant capacity can be mobilized to prevent the ROS level from reaching the cell-death threshold. In contrast, cancer cells show a shift to high ROS generation and elimination, maintaining ROS levels close to the cell-death threshold. Therefore, they would be more dependent on the antioxidant system and more vulnerable to further oxidative stress induced by ROS-generating agents [8]. However, cancer cells may trigger a redox adaptive response leading to anticancer agent resistance. Thus, the potential of these therapies becomes limited. To address this issue, a therapeutic strategy that combines drugs that induce ROS generation with compounds that target the cellular redox adaptive mechanisms was

Abbreviations: ATO, arsenic trioxide; CeO₂, cerium oxide; CNTs, carbon nanotubes; GO, graphene oxide; MNPs, magnetic nanoparticles; MWCNTs, multi-walled carbon nanotubes; Nanoceria, CeO₂ nanoparticles; Nanoprodrug, nanometer-sized prodrug; NPs, nanoparticles; PCL, poly(ϵ -caprolactone); PCL-SS-PEEP, single disulfide bond-bridged block polymer of poly(ϵ -caprolactone) and poly(ethylene glycol); PEG, poly-ethylene glycol; ROS, reactive oxygen species; SOD, superoxide dismutase; S-S, N,N'-bis(acryloyl)cystamine containing the cleavable disulfide-bond; SWCNTs, single-walled carbon nanotubes; TiO₂, titanium dioxide; TiO₂-1, uncoated nano-sized TiO₂ (hydrophilic); TiO₂-2, stearic acid-coated nano-sized TiO₂ (hydrophobic); TPGS, D- α -tocopheryl PEG 1000 succinate; ZnPP, zinc protoporphyrin.

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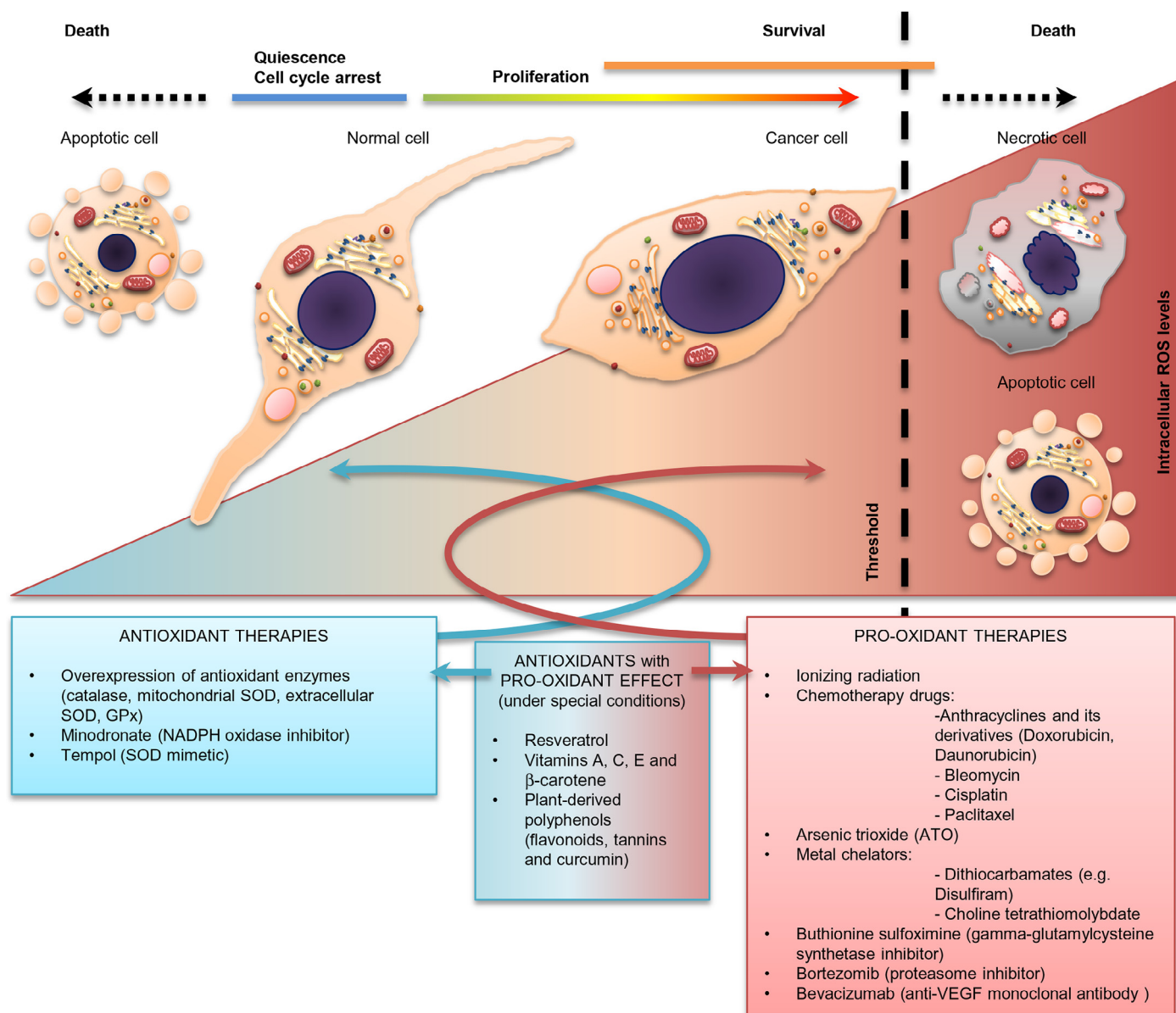


Fig. 1. Anti-cancer therapies based on ROS modulation. The scheme shows the fate of cancer cells exhibiting high levels of ROS that can be altered by anti- or pro-oxidant agents in order to reacquire growth control of normal cells or induce death by apoptosis or necrosis. Some antioxidants which might become pro-oxidants under special conditions are also listed.

proposed [8]. The upregulation of thiol-based antioxidants glutathione, thioredoxin and peroxiredoxin is probably the biochemical basis of redox adaptation and is also involved in drug resistance, being these molecules, as well as other antioxidant enzymes, potential targets for these combined therapies [5, 8]. A more detailed research on the effect of drugs that affect cancer cell oxidative metabolism will help to define better-tailored therapies, decreasing side effects and propensity to develop drug resistance [5].

In order to overcome the limiting tumor hypoxia on cancer therapy, hypoxia-selective redox drugs are also in study (Fig. 2) [9].

Redox therapies based on nanostructures

Nanostructures development for cancer diagnosis and treatment has impacted in the field of medicine. Nanomaterials (Fig. 3) have unique properties given their small size and large surface with

high area-to-volume ratio, allowing them high efficiency to bind, absorb and carry compounds such as drugs and biomolecules. The combination of different functions in nanostructure-based delivery systems enables them to have high stability, targeting capacity, stimuli sensitivity and compatibility with different administration routes, thereby making them highly attractive [56, 57]. Moreover, chemotherapeutics bound as nanoconjugates or encapsulated into nanoparticles cannot be recognized as substrates by the ATP-binding cassette efflux systems, thus evading this drug-resistance mechanism. Nanomedicines to overcome resistance were reviewed in Ref. 58. Approved/commercialized nanomaterials/nanomedicines for cancer treatment and detection as well as others at the various stages of clinical trials are reviewed in Nazir et al. [59].

Different nanomaterials exhibit intrinsic redox properties or may be assembled in complexes with drugs or enzymes for this purpose.

Table 1
Pro-oxidant and antioxidant agents evaluated or used in cancer therapy.

	Type of cancer	Therapeutic effect	Clinical use	References
Pro-oxidant agent				
Arsenic trioxide (ATO)	Acute promyelocytic leukemia	Cell growth inhibition and apoptosis induction. Generation of ROS and impairment of mitochondrial respiration.	Yes	[10–12]
ATO in combination with the antibiotic thiostrepton	Melanoma	Intracellular ROS increase.	To date, it has not been approved for human use	[13]
ATO in combination with disulfiram or buthionine sulfoximine	Melanoma	Apoptosis induction. Pro-oxidant synergistic effect with modulation of glutathione metabolism.	To date, it has not been approved for human use	[14, 15]
Choline tetrathiomolybdate (ATN-224)	Solid tumors, multiple myeloma and chemoresistant hematological malignancies due to oxidative stress resistance or upregulation of Bcl-2	Decrease of cytoplasmic superoxide dismutase (SOD) activity, increase of intracellular oxidants, peroxynitrite-dependent cell death induction. Mitochondrial dysfunction.	Tested in clinical trials for solid tumors and multiple myeloma	[16, 17]
Elesclomol (STA_4783)	Metastatic melanoma	Pro-apoptotic activity. Induction of ROS and oxidative stress.	Phase III clinical trials of elesclomol were suspended in 2008 due to safety concerns	[18]
Bortezomib	Hereditary leiomyomatosis renal cell carcinoma	Proteasome inhibition. Induction of apoptosis and ROS increase.	To date, it has not been approved for human use	[19]
Bevacizumab	Colorectal carcinoma, renal cancer, lung cancer, metastatic breast cancer and ovarian cancer	Monoclonal antibody anti-vascular endothelial growth factor (VEGF). Increase of the oxidative stress marker 8-hydroxy-2'-deoxyguanosine (8-OHdG).	Approved for metastatic colorectal and kidney cancer, advanced nonsquamous non-small cell lung cancer and glioblastoma. Under evaluation for ovarian cancer	[20–25]
Paclitaxel (Taxol)	Unresponsive breast, ovary, and non-small cell lung carcinoma	Impairment of mitosis during the G2/M phase of the cell cycle. Downregulation of protein UCP2 and subsequent induction of ROS linked to the activation of the cell death machinery.	Yes	[26, 27]
Anthracyclin derivatives (doxorubicin and daunorubicin)	Hodgkin's disease, non-Hodgkin's lymphomas, acute leukemias, bone and soft-tissue sarcoma, neuroblastoma, Wilm's tumor, and malignant neoplasms of the bladder, breast, lung, ovary, and stomach	Inhibition of both DNA replication and RNA transcription. Free radical generation leading to DNA damage or lipid peroxidation, DNA cross-linking, DNA alkylation, direct membrane damage due to lipid oxidation and inhibition of topoisomerase II.	Yes	[28–31]
Antioxidant agent				
Overexpression of antioxidant enzymes, such as catalase, mitochondrial SOD, extracellular SOD, glutathione peroxidase	Experimental tumors	ROS scavenging	To date, it has not been approved for human use	[32–44]
Minodronate	Experimental tumors and bone metastases from breast cancer	Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase inhibition. Impairment of the VEGF signaling suppressing endothelial ROS generation, probably via inhibition of geranylgeranylation of Rac.	Adjuvant use of bisphosphonates in phase III clinical trials in patients with early breast cancer	[45–48]
4-Hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (4-hydroxy-TEMPO or Tempol)	Experimental tumors	SOD mimetic catalyzing the disproportionation of O ₂ ^{•-} . Inhibition of cell proliferation and apoptosis induction.	To date, it has not been approved for human use	[49]
3,4',5-Trihydroxystilbene (resveratrol)	Experimental tumors, breast, cancer, colorectal cancer and lymphoma	Antioxidant effect. Inhibition of ROS-induced DNA damage. On the other hand, pro-oxidant effect in the presence of transition metal ions such as copper.	Under evaluation in ongoing trials in colon cancer and lymphoma	[50–52]
Curcumin	Experimental tumors, colorectal cancer, pancreatic cancer and multiple myeloma	Phenolic chain-breaking antioxidant, donating H-atoms from the phenolic group	Phase I/II trials are going on for colorectal and pancreatic cancers as well as multiple myeloma	[53–55]

Besides, their potential to improve delivery and target selectivity may limit side effects. These features contribute to make them even more interesting for cancer treatment.

Herein, we will describe the state of the art regarding redox therapies based on nanostructures.

Pro-oxidant nanostructures

Among drug delivery systems, liposomes [60–62] play a key role and several formulations were approved for use or are in clinical trials [63]. Actually, for cancer therapeutics, they have evolved from

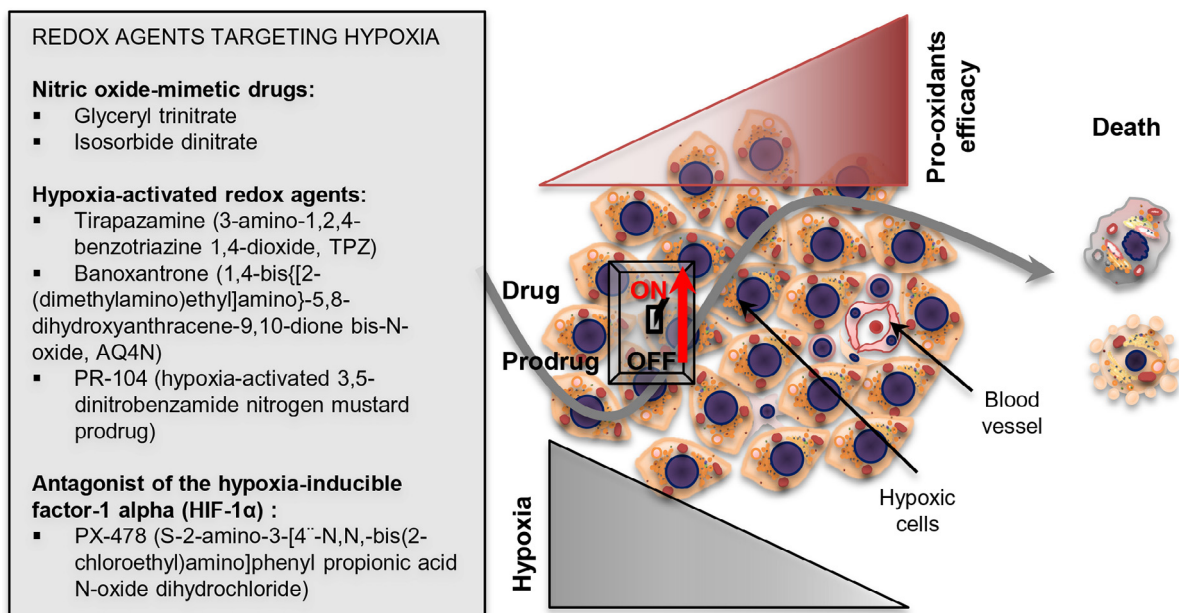


Fig. 2. Redox therapies under development to overcome tumor hypoxia. Tumors develop hypoxic conditions by insufficient tumor vascularization and oxygenation, which can select genetic alterations and metabolic adaptations that favor tumor progression under adverse conditions of limited oxygen supply. Tumor hypoxia is often associated with resistance to treatments and represents a phenotypic alteration characteristic of tumor tissue and is therefore an important drug target for various classes of anticancer therapeutics currently in preclinical and clinical development, reviewed in Wondrak [9]. Low concentrations of nitric oxide-mimetic drugs overcome hypoxia-induced drug resistance through activation of nitric oxide signaling. Moreover, nitric oxide mimetics exerted potent chemosensitization to doxorubicin in a mouse xenograft model of human prostate cancer. Bioreductive activation of hypoxia selective prodrugs (tirapazamine, banoxantrone and PR-104) initiates the formation of cytotoxic free radical and other reactive intermediates that kill cells under hypoxic conditions, but spare cells under conditions of normoxia due to oxygen-dependent electron transfer reactions that lead to inactivation of the cytotoxic free radical intermediate. In clinical trials, tirapazamine has displayed modest therapeutic efficacy that could be improved based on careful phenotyping of tumors with clinical parameters characteristic of hypoxia. Banoxantrone (AQ4N) and PR-104 were also evaluated in Phase I/II clinical trials for solid tumors alone or in combination with radiotherapy and/or chemotherapeutic drugs.

conventional to second generation, such as dual-drug loaded or stimuli-sensitive liposomes, reviewed in Ref. [64]. Surface modified liposomes were studied as nanocarriers for tumor-targeted and intracellular delivery [3, 65–67]. However, examples of liposomes encapsulating drugs for ROS generation and cytotoxicity in tumor cells are scarce. The selective delivery of doxorubicin to increase ROS levels and cytotoxicity by using dual-ligand surface-modified liposomes in human oral carcinoma cells was described. Folic acid and triphenylphosphonium cations were used in this system as ligands for cancer cell and mitochondria targeting, respectively [68]. The oxidative and chemotherapeutic effect of liposomal doxorubicin combined with radiofrequency-thermo ablation was reported [69]. Besides, sensitization of breast cancer cells to circumvent multidrug resistance and enhance therapeutic response was demonstrated with doxorubicin–liposomes–microbubble complexes assisted by ultrasound [70]. Anatase particles of titanium dioxide (TiO_2) can absorb ultraviolet light and photoexcited TiO_2 is a strong oxidizer. Liposome-encapsulated TiO_2 improved its rapid internalization into target cells and enhanced anti-tumoral effect in an *in vivo* bladder cancer model [71].

In contrast, there are several examples of nanoparticles (NPs) as drug delivery systems for ROS generation with anticancer effects including hydroxyapatite NPs [72], aminosilanized oxidized silicon NPs [73] and X-ray irradiated gold NP functionalized with doxorubicin conjugated to DNA strands [74]. Nano-sized TiO_2 coated with stearic acid (TiO_2 -2, hydrophobic) generated high levels of ROS in cell-free conditions. TiO_2 -2 elicited a strong response, resulting in cytotoxicity to fibrosarcoma cells [75]. High doses of iron-oxide NPs may be used to generate an oxidative assault for cancer therapy [76]. The prooxidant effect of tetrandrine amplifies the antitumor effect of paclitaxel. Biodegradable core-shell methoxy poly-ethylene glycol-

polycaprolactone NPs containing tetrandrine and paclitaxel showed synergistic antitumor effect against gastric cancer cells [77].

Polymeric micelles are formed of block copolymers, which assemble in aqueous solution as outer hydrophilic layer and inner hydrophobic core [78]. These nanostructures can provide an excellent advantage of smaller size in comparison to liposomes [79]. Polymeric micelles of zinc protoporphyrin (ZnPP) with poly-ethylene glycol (PEG) or styrene maleic acid copolymer demonstrated selective targeting to tumor tissues based on the enhanced permeability and retention effect. The anticancer activity was a consequence of the heme oxygenase-1 inhibitory potential. Furthermore, ZnPP efficiently generated reactive singlet oxygen, which may also favor ROS-induced tumor cell death [80, 81]. Palmitoyl ascorbate-loaded polyethylene glycol–phosphatidylethanolamine micelles exhibited anti-cancer activity both *in vitro* and *in vivo* due primarily to ROS generation [82]. Superparamagnetic iron oxide NPs-micelles improved β -lapachone anticancer efficacy by amplifying ROS production [83].

Carbon nanotubes (CNTs), single-walled (SWCNTs) or multi-walled (MWCNTs), which can penetrate cell membranes, were explored as drug delivery carriers [84, 85]. SWCNTs and graphene oxide (GO) were combined for potentiating the efficacy of paclitaxel for lung cancer treatment, showing enhanced cell death by a synergistic and ROS dependent effect [86]. Regarding photodynamic therapy, human lung cancer cells exposed to high-density lipoprotein-stabilized semiconducting SWCNTs and near-infrared laser irradiation exhibited enhanced cell death induced by singlet oxygen generation [87].

Antioxidant nanostructures

Some nanomaterials and nanostructures exhibit antioxidant properties, which may also be considered an advantage for cancer therapy.

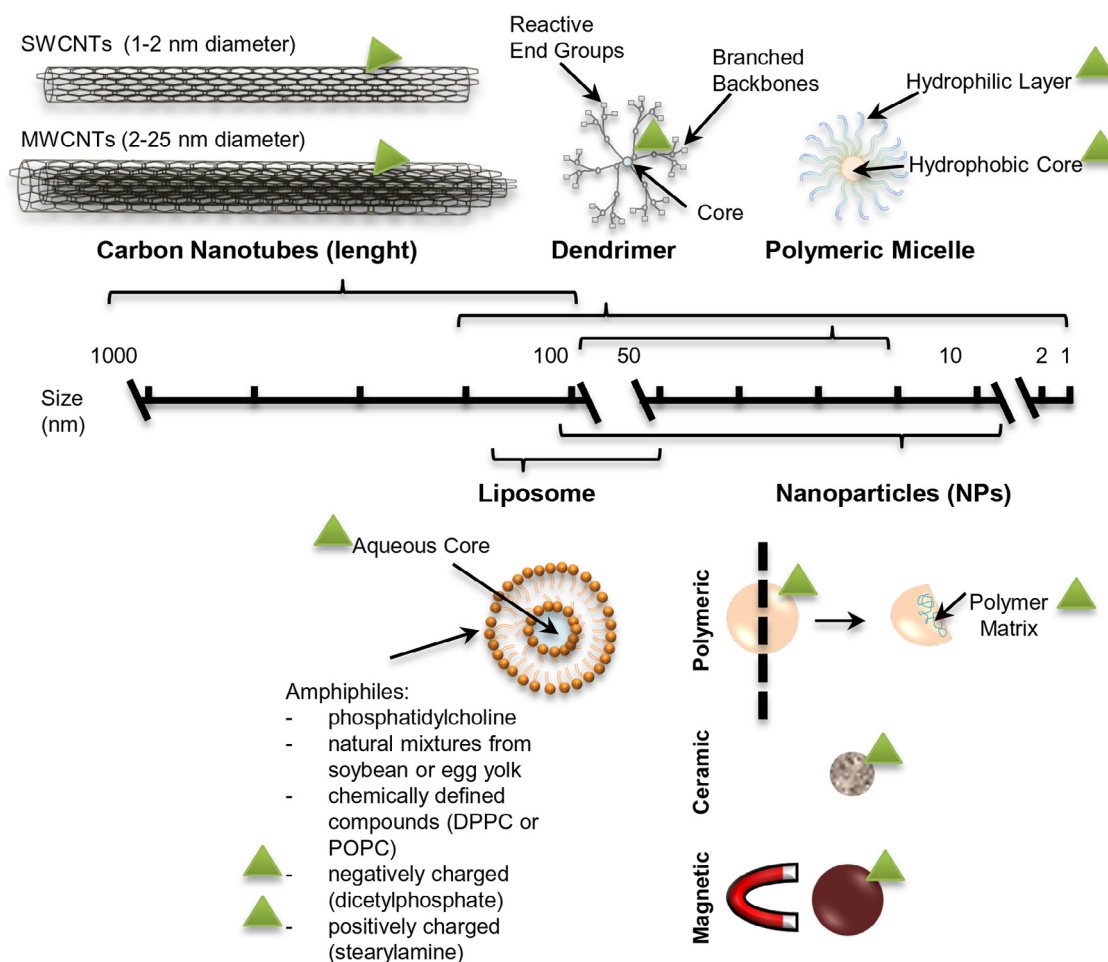


Fig. 3. Conventional nanomaterials evaluated for cancer treatment. Curly brackets indicate the size range. Arrows show the different components of the nanomaterials and green triangles represent the localization of the therapeutic agents or ligands that allow the targeted delivery. Under osmotically balanced conditions, liposomes contain one or more lamellae that are composed of the amphiphiles. Depending on the method of preparation, liposomes can be multi-, oligo- or unilamellar, containing many, a few, or one bilayer shell(s), respectively [60–62]. DPPC: 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; POPC: 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine. Nanoparticles (NPs) include polymeric NPs, ceramic NPs, magnetic NPs (MNPs), dendrimers and polymeric micelles [78]. Polymeric NPs are small polymeric colloidal particles with a therapeutic agent either dispersed in the polymer matrix (nanosphere) or encapsulated in the polymer (nanocapsule). Ceramic NPs are interesting drug-delivery vehicles due to their ultra-low size (less than 50 nm) and possible porous nature. These NPs do not show swelling or changes in porosity with pH. Thus, they can protect proteins or enzymes against denaturation induced by changes in the external pH and temperature. Silica and other materials such as titania, which are highly compatible with biological systems because of their inert nature, are also widely being used for the formulation of these NPs. MNPs are nanometer-sized ferrite- or magnetite (Fe_3O_4)-based spherical particles. MNPs can be coated with various hydrophilic polymers for stability and can be loaded with therapeutic agents. Because of their small volume, MNPs usually show superparamagnetic behavior, meaning that the magnetic moment of each MNP will be able to rotate randomly (in reference to the orientation of the MNP) just because of the temperature influence. This property, marked by the lack of residual magnetization after removal of external fields, enables the MNPs to maintain their colloidal stability and avoid agglomeration [139]. Dendrimers, a class of synthetic polymers, are monodispersed symmetric macromolecules with a well-defined three-dimensional structure consisting of highly branched backbones built around a small molecule with an internal cavity surrounded by a large number of reactive end groups. The size and shape of dendrimers depend on their number of branched units (i.e., generation) and their backbone constituents. Polymeric micelles are formed of block copolymers. Water-insoluble therapeutic agents can be loaded into the hydrophobic core of the micelles. They can also be conjugated with different ligands and antibodies to achieve site-specific targeting. Carbon nanotubes (CNTs) can either be single-walled (SWCNTs) or multi-walled (MWCNTs) [84]. The chemistry of CNTs and, in particular, the high aspect ratio (i.e., length/width) that leads to a large surface area offers the possibility of introducing more than one function on the same tube (targeting molecules, contrast agents, drugs, or reporter molecules can be used at the same time) [142].

Antioxidant enzymes were proposed for the treatment of several pathologies where oxidative stress is involved. Different strategies to improve antioxidant enzyme delivery have been reported. For example, chemical modification techniques have been used to control the tissue distribution of catalase [88]: galactosylation, mannosylation or succinylation, cationization and PEGylation [38]. Significant inhibition of the ROS-mediated oxidative tissue damage and decreased expression of ROS-responsive genes associated with metastatic growth of tumor cells were observed [88]. Moreover, the inhibition of spontaneous lung metastasis of melanoma cells in mice by gelatin hydrogel sheets from which PEGylated catalase is gradually released was demonstrated [34]. Similarly, intravenous injection

of PEGylated-superoxide dismutase (SOD) inhibited peroxidation and melanoma cell spontaneous lung metastasis in mice [89].

Since the preparation of lysozyme-containing lipid vesicles was reported [90], different enzymes were entrapped in liposomes. Some of them were summarized and selected by their potential use in biomedical applications [62], such as asparaginase as antitumor agent in lymphoblastic leukemia and DNA-lyase for repairing UV-induced DNA damage and skin cancer treatment among others. Liposomes as an antioxidant delivery system were described containing enzymatic, such as SOD and catalase, or non-enzymatic antioxidants, like glutathione and tocopherols [91]. Antioxidant liposomes for vascular delivery were reviewed [92]. Regarding antioxidant enzyme

encapsulation, one of the first reports showed intracellular delivery of liposomal SOD to aortic endothelial cells in an oxygen sensitive lung cell type, protecting these cells from oxygen induced damage [93]. Other liposomal antioxidants include polyphenolic compounds, such as curcumin [94], resveratrol and quercetin. Liposomal curcumin exhibited tumor growth inhibition and antiangiogenic effects in a xenograft human pancreatic cancer model [95]. The liposomal delivery of curcumin and resveratrol reduced the incidence of prostate cancer in a tumor suppressor gene knockout mouse model [96]. Quercetin encapsulated in PEGylated liposomes showed enhanced antitumor activity against lung, colon and hepatic cancer in murine models [97].

Artificial enzymes were used to promptly eliminate excessive ROS in the cells [98, 99]. Peptides, cyclodextrin, graphene and polymers with catalytic active units were developed as artificial enzymes and metal and/or metal oxide NPs were reported to efficiently decrease ROS levels [99–106]. Although encapsulated peptides or proteins in NPs are immunogenic and improvements in their catalytic activities are needed [99, 107], promising developments of compounds or NPs with antioxidant enzyme mimetic activities were reported. For example, Fe₃O₄ and cobalt oxide NPs possess peroxidase-like and catalase-like activities [105, 106]. Apoferritin-encapsulated platinum NPs are able to efficiently quench H₂O₂ and O₂^{•-} [100]. Apoferritin-encapsulated cerium oxide (CeO₂) NPs can act as SOD mimetics, in which apoferritin can manipulate the electron localization on the surface of CeO₂ NPs and improve their ROS scavenging activities [102]. The NPs of CeO₂ (nanoceria) catalytically remove O₂^{•-} and potentially other ROS and reactive nitrogen species, being the main molecular mechanism behind the antioxidant properties of these NPs [108]. Nanoceria were also proposed as anti-angiogenic in ovarian cancer [109]. The antioxidant capabilities of nanoceria were also explored for radiation protection and the potential treatment of other ROS related disorders, such as diabetes and macular degeneration [110]. Furthermore, nanoceria revealed an inhibitory effect on the formation of myofibroblasts. Besides, concentrations of redox-active polymer-coated nanoceria being non-toxic for normal stromal cells showed a ROS-dependent cytotoxic and anti-invasive effect on squamous tumor cells and melanoma cells *in vitro* and *in vivo* [111, 112]. In order to improve the synthesis of artificial antioxidant enzymes, acetylated generation 9 polyamidoamine dendrimers encapsulated in platinum NPs mimic catalase showing similar size, globular shape and catalytic activity as compared to the enzyme [99].

Recently, the inhibition of melanoma cell proliferation by MNP-catalase nanocomplexes was shown. The system synergically exploited both the ability of catalase to scavenge cell-generated H₂O₂ and magnetite manipulation to modulate cell proliferation between arrest and growth using magnetic fields [113].

Poloxamers and D- α -Tocopheryl PEG 1000 succinate (TPGS) are known inhibitors of P-glycoprotein, which is overexpressed in multidrug resistant cells. Mixed polymeric micelles prepared from Poloxamer 407 and TPGS containing the antioxidant and anticancer agent gambogic acid showed cellular uptake and enhanced cytotoxicity on breast cancer and multidrug resistant cells [114].

The flavonoid catechin, a green tea antioxidant, exhibits cancer chemopreventive effect against a broad spectrum of invasive cancers. Non-covalent incorporation of CNTs into a gelatin–catechin covalent conjugate showed a considerable increase in the therapeutic activity of this flavonoid in HeLa cancer cells [115].

Nanotechnological strategies based on redox state of tumor microenvironment

Studies on tumor microenvironment are relevant to design new targeted therapies [116] based on its differences with normal tissue including vascular abnormalities, oxygenation, perfusion, pH,

metabolic and redox states. In this regard, pH-responsive drug delivery nanosystems were developed to enhance drug release in response to acidic tumor microenvironment [117]. In relation to tumor vasculature, the enhanced permeability and retention effect is considered in the design of cancer-targeting drug nanocarriers as a guiding principle [116].

Different strategies were proposed to take advantage of the oxidative tumor microenvironment. An example is nanometer-sized prodrug (nanoprodrug) of camptothecin to treat experimental glioblastoma multiforme based on the greater activation of the nanoprodrug when it is oxidized [118].

Considering that cancer cells exhibit increased amounts of ROS, H₂O₂-responsive nanocarriers self-assembled from an amphiphilic hyperbranched polymer consisting of alternative hydrophobic selenide groups and hydrophilic phosphate segments in the dendritic backbone were developed as anticancer agent. The constructed nanocarrier can be disassembled under an exclusive oxidative microenvironment within cancer cells resulting in rapid and selective intracellular drug release, because the amphiphilic hyperbranched polymer becomes hydrophilic after oxidation [119].

Another interesting targeted cancer therapy approach is the development of dual and multi-stimuli responsive polymeric NPs with programmed site-specific drug delivery feature [120]. Polymeric NPs that degrade upon exposure to two stimuli in tandem, ROS and low pH, were designed [121]. Although aimed for targeting inflammation, these NPs have potential for cancer treatment considering the resemblance of both inflammatory and tumor microenvironments.

On the other hand, differences between the intracellular and the extracellular environments of some ROS were also exploited for preparing stimuli-responsive drug nanocarriers. This applies to glutathione, a free radical scavenger that protects cells from harmful effects of ROS, toxins, drugs and many mutagens [122–124]. While glutathione deficiency, or a decrease in the glutathione/glutathione disulfide ratio, leads to an increased susceptibility to oxidative stress implicated in the progression of cancer, elevated glutathione levels increase the antioxidant capacity and the resistance to oxidative stress as observed in advanced stages of many cancers [122]. Moreover, multidrug resistance in cancer cells is often associated with an increased concentration of reductive glutathione [125, 126]. The disulfide functional group has gained attention in the preparation of stimuli-responsive drug carriers because of its stability in mildly oxidizing environments and its liability in the presence of reducing agents [124, 127]. Because of the large redox potential difference between the extracellular matrix (thiol concentration: 10–40 μ M) and the cytosol of cancer cells (thiol concentration: 0.5–10 mM because of the presence of glutathione) [128], the reversible disulfide thiol conversion is being widely used for cytosolic drug delivery [124]. Redox-responsive nanocarriers were generated in order to take advantage of the reductive intracellular environment of resistant cancer cells. A micellar nanodrug carrier assembled from the single disulfide bond-bridged block polymer of poly(ϵ -caprolactone) and poly(ethylene phosphate) (PCL-SS-PEEP) loaded with doxorubicin was developed. The shell detachment of the PCL-SS-PEEP NPs caused by the reduction of intracellular glutathione significantly accelerated the drug release. This nanocarrier significantly enhanced the cytotoxicity of doxorubicin to multidrug resistance breast cancer cells, which renders the redox-responsive NPs a promising strategy for cancer therapy [129]. In a similar approach, doxorubicin-loaded polyphosphate nanosized assemblies based on amphiphilic hyperbranched multiarm copolyphosphates with redox-responsive backbone self-assembled into spherical micellar NPs were designed. These redox-responsive micelles exhibited a fast glutathione-mediated intracellular delivery of the anticancer drug into the nuclei of glutathione monoester pretreated HeLa cells enhancing the inhibition of cell proliferation [130]. Single mature caspase-3 was encapsulated in a polymeric nanocapsule, which can

reversibly release the protein in the reducing environment of the cytosol. Caspase-3 was mixed with acrylamide, positively-charged N-(3-Aminopropyl) methacrylamide and the crosslinker. *In situ* polymerization was initiated by the addition of free radical initiators after the monomers were electrostatically adsorbed onto the caspase-3 surface. To render the crosslinking of the capsule reversible under reducing conditions, N,N'-bis(acryloyl)cystamine containing the cleavable disulfide-bond (referred to as S–S) was used. Caspase-3 delivered using S–S nanocapsules was able to induce apoptosis in HeLa, MCF-7 and U-87 MG cancer cells [131]. Folate conjugated, disulfide-crosslinked, polymer-coated, and acoustically reflective lipid NPs were developed for cytosolic drug delivery. These NPs were stable in the extracellular oxidizing environment but released their contents efficiently in the reducing environment of cell cytosol. This release was further enhanced by applying diagnostic frequency ultrasound. The folic acid led to enhanced uptake and cytotoxicity of these doxorubicin-loaded lipid NPs in cancer cells overexpressing the folate receptor. With further developments, these NPs could have the potential to be used as multimodal nanocarriers for simultaneous targeted drug delivery and ultrasound imaging [124].

In cancer diagnostics, hydrocyanine-conjugated chitosan-functionalized pluronic-based nanocarriers were synthesized for selective imaging of ROS in tumor sites. The reduction of cyanine to hydrocyanine of the nanocarriers resulted in complete disappearance of fluorescence emission, and the fluorescence recovered by ROS-induced reoxidation. Hydrocyanine-nanocarriers could detect various ROS including $O_2^{\bullet-}$ and hydroxyl radical in a dose-dependent manner, being stable in serum-containing media and not showing acute cytotoxicity. The selective *in vivo* near infrared fluorescence image of the tumor sites was obtained without background signals from non-specific localization of the probes [132].

Fig. 4 summarizes the different strategies described herein based on redox nanomaterials for cancer therapy.

The dark side of nanostructures and redox therapies

Although nanotechnology could improve cancer treatments, the side effects of nanostructures must be considered. Caution should be taken in their fabrication, handling, and disposal [75, 133]. These newly synthesized nanomaterials can therefore have double-bladed effects; while they can be beneficial to health, they can also have deleterious repercussions that were not anticipated at their designing and engineering stages [75].

NP-mediated ROS responses have been reported to orchestrate a series of pathological events such as genotoxicity, inflammation, fibrosis, and carcinogenesis [134]. For example, poorly tumorigenic and non-metastatic fibrosarcoma cells became tumorigenic and even metastatic after injection into sites previously implanted with uncoated nano-sized TiO_2 (TiO_2 -1, hydrophilic), but not with stearic acid-coated (TiO_2 -2, hydrophobic). Although both TiO_2 -1 and TiO_2 -2 resulted in intracellular ROS formation, TiO_2 -2 elicited a stronger response, resulting in cytotoxicity to the fibrosarcoma cells [75]. Moreover, intravenous injection of TiO_2 NPs at high doses in mice induced acute toxicity in the brain, lung, spleen, liver, and kidney [135]. Spinel ferrite NPs with the general formula MFe_2O_4 (where M is +2 cation of Ni, Mn, Zn or Co) exhibit interesting magnetic and electrical properties with good chemical and thermal stabilities. These nanocrystalline materials are used in many applications including hyperthermia for cancer treatment. Nickel ferrite NPs induced cytotoxicity in a dose-dependent manner in the concentration range of 25–100 $\mu\text{g}/\text{ml}$ in human lung epithelial cells. These NPs triggered apoptosis through ROS generation and glutathione depletion. Further investigation is required to determine the *in vivo* toxicity of nickel ferrite NP application [136]. MNPs at high doses induced DNA strand breaks directly or indirectly through oxidative DNA

damage, followed by clastogenic events including micronuclei formation and sister chromatid exchange [137, 138]. These findings must be taken into account for the therapeutic use of MNPs in cancer and caution must also be taken in their use and manipulation due to the potential genotoxicity of these particles. Some MNPs manufactured for its application as magnetic resonance imaging contrast agents have been currently removed from the market due to potential *in vivo* toxicity. Severe back, groin, leg or other pain, or allergic reactions were reported as side effects of some of these contrast agents due to the degradation and clearance of MNPs from circulation by the endogenous iron metabolic pathways. It is not currently known if MNPs have a risk of tumorigenesis. Therefore, studies dealing with the long term influence of MNPs in the organism are highly required [139].

The immunogenicity of dendrimers may cause unwanted systemic immune reaction which may limit their therapeutic application [140]. A potential relative advantage of 5 nm-dendrimer nanocomposites in delivering therapeutic agents to tumors was proposed because there may be less immune recognition [141].

Pristine, water-insoluble CNTs have been found to be highly toxic *in vitro* to many different types of human cells [142–145]. CNTs have been shown to promote the aggregation of human platelets *in vitro* [146] and to induce the formation of lung granulomas in mice [147]. Moreover, exposure of mesothelial cells to raw SWCNTs resulted in the generation of hydroxyl radical, leading to several molecular alterations associated with carcinogenesis. Such responses were similar to reported asbestos-induced changes common in animal and human mesothelioma development. However, *in vivo* animal studies are warranted to address whether SWCNT exposure is a risk for mesothelioma development in humans [148].

Regarding redox therapies, increased ROS may induce toxicity. In this sense, free radicals produced by oncologic therapies are often a source of serious side effects, which are the main limitation of treatments, depending on its cumulative dose. In the same way, the administration of all traditional antineoplastic drugs is accompanied by adverse reactions arising from the limited selectivity of their anticancer action. For example, in the case of doxorubicin, one of the proposed mechanisms of cardiac dysfunction is an oxidation of cellular components via formation of ROS [28]. Considering this, some data suggest that antioxidants can ameliorate toxic side effects of therapy without affecting treatment efficacy, whereas others suggest antioxidants interfere with radio- and chemotherapy which are largely dependent on ROS to induce cytotoxicity in tumors [4]. As with other novel experimental therapies that enter clinical trials, redox-based agents with good efficacy in cell culture and in animal models may pose obstacles related to different parameters, such as pharmacokinetics, selectivity and systemic toxicity, during later stages of development that should be further considered [9].

Conclusion and future perspectives

Herein, we presented an overview of recent advances in nanomaterials research in the context of current and emerging ROS-modulated strategies for cancer therapy. Considering that latest advances in material engineering geared toward biomedical applications have involved the tuning of nanomaterials to incorporate several functions, a straightforward combination of redox nanosystems with different components, such as fluorophores and magnetically susceptible domains will allow developing novel multifunctional nanovehicles. The evaluation of the redox status of different types of cancer becomes relevant as this could enable the design of a new generation of redox-active nanomaterials for increasing the selectivity and targeting of cancer therapies. Based on several pieces of evidence, possible side effects of nanomaterials administration and redox therapies should be considered in order to achieve a proper and better treatment. Taking into account that

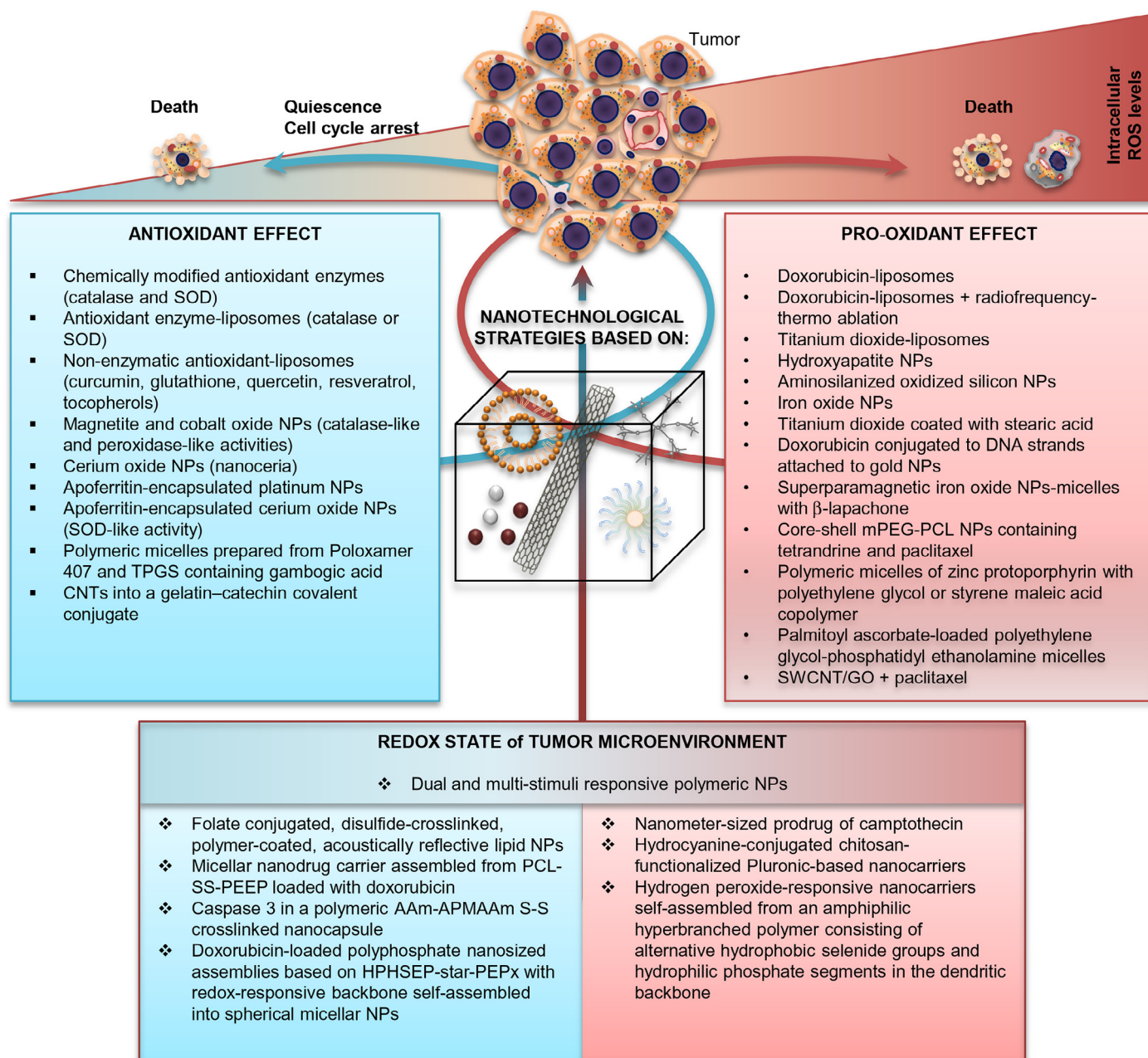


Fig. 4. Anti-cancer redox therapies based on nanostructures. The scheme shows the fate of a tumor when it is exposed to nanostructures with antioxidant/pro-oxidant effect or to nanodrugs activated or selectively delivered by redox state of tumor microenvironment in order to inhibit growth or induce death by apoptosis or necrosis.

current strategies against cancer are aimed to potentiate their effects through the combined use of diverse agents with different mechanisms of action, the nanostructure multifunctionality in association with conventional treatments as well as redox modulation approaches could lead to improve cancer management.

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Conflict of interest

The authors declared that there is no conflict of interest.

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