Nanomedicine-driven molecular targeting, drug delivery, and therapeutic approaches to cancer chemoresistance

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Cancer cell resistance to chemotherapeutics (chemoresistance) poses a significant clinical challenge that oncology research seeks to understand and overcome. Multiple anticancer drugs and targeting agents can be incorporated in nanomedicines, in addition to different treatment modalities, forming a single nanoplatform that can be used to address tumor chemoresistance. Nanomedicine-driven molecular assemblies using nucleic acids, small interfering (si)RNAs, miRNAs, and aptamers in

combination with stimuli-responsive therapy improve the pharmacokinetic (PK) profile of the drugs and enhance their accumulation in tumors and, thus, therapeutic outcomes. In this review, we highlight nanomedicine-driven molecular targeting and therapy combination used to improve the 3Rs (right place, right time, and right dose) for chemoresistant tumor therapies.

Introduction

Chemoresistance refers to the resistance of tumor cells and/or tissues to drugs and is usually a multidrug resistance (MDR) phenomenon. It continues to be a leading cause of poor survival and disease relapse in almost all types of cancer. Chemoresistance occurs as a result of changes within tumor cells, with the tumor microenvironment (TME) also influencing drug resistance mechanisms [1–3]. Multiple biomolecular mechanisms are involved in the development of chemoresistance in cancer cells, including, but not limited to, overexpression of drug efflux transporter

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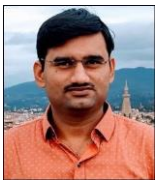

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pumps (e.g., P-glycoprotein; P-gp), augmented DNA repair activities, cancer stem cells (CSCs), and dysregulation of apoptosis [2]. Chemoresistance takes many forms, including insufficient drug entry into the TME and cells, limited drug accumulation in the tumor, increases in drug efflux, and enhanced DNA repair [4]. The basics of chemoresistance, including the molecular mechanisms, pathologies, and clinical concepts, have been reviewed elsewhere [3,5,6]. Thus, we focus here on molecular drug delivery approaches coupled with nanomedicines, which have been extensively evaluated to combat chemoresistance in recent years.

Current cancer nanomedicines have the potential to overcome chemoresistance at the clinical stage by enhancing the accumulation of chemotherapeutics in tumors using an optimal molecular targeting approach, resulting from the multifunctionality of nanoparticles (NPs). Thus, new-generation nanomedicines represent an innovative and promising therapeutic approach to overcome the various limitations of conventional chemotherapeutics, based on localized, enhanced, and selective drug accumulation using molecular targeting [7]. Nanomedicine-driven molecular drug targeting (e.g., installing ligands on the surface of nanocarriers) allows optimal dosing within the therapeutic window; a crucial aspect for the success of a targeted therapy against chemoresistant tumor tissues. Contextually, it modulates the therapeutic index (drug selectivity, tumor characteristics, etc.) target selectivity, and resistance liabilities [8,9] (Box 1).

Here, we explore recent advances in nanomedicine-driven molecular targeting methods and present a future perspective on the advantages and limitations of this novel platform developed to overcome well-defined mechanisms of cancer chemoresistance.

Chemoresistance: concept and current clinical scenario

Chemoresistance is either an intrinsic phenomenon of the tumor or can develop during treatment (Fig. 1a). Given the unpredictable nature of tumor cells and their molecular heterogeneity, patients with cancer treated with chemotherapeutic drugs can eventually develop resistance against these agents. Cancerous tumors comprise a basement membrane, vasculature, immune cells, and TME; in addition, pathological heterogeneity, physical parameters, the genome, and surrounding environment of the tumor contribute to chemoresistance. There are also other physiological principles are associated with chemoresistance. These physical factors, such as extracellular matrix (ECM), interstitial fluid pressure, the hypoxic core, and the extracellular pH of tumors, contribute to tumor chemoresistance by acting as a physical barrier against drug intake. Recent advances have also highlighted other factors contributing to chemoresistance, including: (i) hypoxia, pH, and glucose concentration in the tumor; (ii) tumor heterogeneity; (iii) immune system and tumor microenvironment; (iv) undruggable genomic drivers; and (v) miRNA-mediated chemoresistance.

Nanomedicine-driven molecular targeting: concept

Current oncological protocols used to tackle chemoresistance rely on low-molecular-weight (LMW) drugs. Chemotherapeutics are generally <1 nm, whereas nanomedicines (NPs + chemotherapeutics + targeting molecules) are 1–2 orders of magnitude larger than conventional drugs or anticancer molecules. Given their larger size, nanomedicines are not only internalized via passive diffusion but also endocytosed via endolysosomal trafficking by drug-resistant cancer cells; thus, they can be localized deep inside the cells [10–12]. Additionally, the endocytosis of nanomedicines provides an opportunity to bypass drug efflux pumps, which is a prominent feature responsible for chemoresistance. In addition to endolysosomal trafficking, cancer cell-specific targeting moieties on nanomedicines have intrinsic characteristics for internalization into chemoresistant tumor cells and the TME. Thus, high localization of nanomedicines along with chemotherapeutic cargo into TME and tumor cells can suppress the overexpression of MDR proteins.

In addition to chemotherapeutic agents, new-generation nanomedicines are designed to combine LMW MDR inhibitors, nucleic acid-based therapeutics, and anti-MDR siRNAs in a single nanoplatform. In recent years, the combination approach of molecular targeting and therapy has been extended to provide synergetic therapies at the advanced clinical stage of chemoresistance cancer management (Fig. 1b, Box 2) [13–18]. These recently developed nanomedicines exhibit multifunctionality, such as: (i) prolonged circulation properties; (ii) ability to accumulate in cancerous tumor via enhanced permeability and retention (EPR) effect; (iii) P-gp activity inhibition; (iv) initiation of cascade reactions that drastically increase the overall effectiveness against MDR tumors and (iv) increased cellular uptake by receptor-mediated endocytosis. Most chemotherapeutics are taken up by cells via a passive diffusion mechanism that is a highly efficient process in cancerous cells. However, in chemoresistant cells, most of these promptly localized drug molecules are rapidly sensed by MDR proteins and efflux from the cells, resulting in chemotherapy failure. By contrast, nanomedicine-driven active molecular targeting ensures efficient delivery and high intracellular concentrations of chemotherapeutic cargoes into chemoresistant cells and TME, making them interesting therapeutics for treating chemoresistant tumors.

Delivery of P-gp-suppressing nanomedicines

Despite significant improvements achieved in the overall survival of patients undergoing conventional cancer therapies, chemoresistance remains a major obstacle to a positive outcome [8]. The application of sequencing technology to the human genome and genetic data compiled from patient tumor samples has revolutionized the use of targeted cancer therapies, such as nanomedicines. Nanomedicine-targeted therapy module provides promising alternatives to conventional passive drug delivery platforms. Active therapeutic delivery can be achieved by chemically conjugating molecular targeting moieties to the nanocarriers. These molecular targeting platforms allow targeted binding in the nanomolar to picomolar range and differentiation between target cancer cells and normal cells, For example, single-stranded DNA or RNA (nucleic acid)-based nanoplatforms specifically identify cancer cells via overexpressed cell surface receptors and deliver their therapeutic cargoes exclusively to malignant cells [19]. Furthermore, nanomedicine-driven molecular targeting can allow high intracellular accumulation of chemotherapeutic cargoes and multiple therapeutic payloads through receptor-mediated endocytosis [20]. The synergic effect of multiple and simultaneous chemotherapeutics delivery could inhibit tumor growth by regulating several different signaling pathways, while minimizing the occurrence of chemoresistance [21].

BOX 1

Chemoresistance

'Chemoresistance' refers to the resistance of tumor cells to various types of drug via different molecular mechanisms. Chemoresistance to chemotherapeutics is classified into intrinsic (i.e., existing since the beginning of therapy) and acquired (i.e., developed during the course of treatment) resistance.

The most advantageous strategy to overcome chemoresistance is to localize effective drug concentrations within all tumor cells using molecular targeting mechanisms that can improve therapeutic responses. Overexpression of P-gp by tumor cells decreases the intracellular accumulation of various small-molecule chemotherapeutics, such as doxorubicin (DOX), paclitaxel (PTX), and cisplatin, which results in lower therapeutic efficacy. Therefore, suppressing P-gp expression has become an effective way to control chemoresistance and enhance chemotherapy efficacy. New-generation nanomedicines, such as nanoliposomal formulations that cam bypass MDR by utilizing the systemic administration of combinatorial siRNAs, DNA-aptamers, and chemotherapeutic drugs, show advantages against chemoresistant cancers [22]. This novel strategy uses polymer-substituted aptamer-grafted lipid NPs (LNPs) with enhanced transfection efficiency and low cytotoxicity to deliver P-gp-specific siRNAs into chemoresistant breast cancer cells. Aptamer targeting facilitates siRNA nanocarriers, which can inhibit P-gp expression more efficiently than nanocarriers without aptamers. Rather than simply increasing local amounts of less-active therapeutic cargos to suppress P-gp, the net, more advantageous effect is to deepen the response by localized delivery of stimuli-responsive chemotherapeutic agents. This was recently demonstrated by the self-assembly of complex amphiphilic drug-inhibitor conjugates (ADIC) containing a redox-responsive linkage for suppressing the chemoresistance of breast cancer cells in clinical settings [23]. Specifically, the hydrophilic anticancer drug irinotecan (Ir) and the hydrophobic P-gp protein inhibitor quinine (Qu) were linked by a redox-responsive bridge. After localization into a tumor, the Qu was released via glutathione (GSH)-triggered cleavage of the disulfide bond, resulting in the downregulation of P-gp expression. This, in turn, prevented Ir from being pumped out, increasing its concentration in drug-resistant cells and ultimately resulting in cancer cell death. Overall, this strategy based on redox-responsive amphiphilic drug–inhibitor conjugates offers a new way to overcome tumor chemoresistance and to improve the success rate of chemotherapy. By contrast, nanomedicine-driven molecular targeting and chemotherapeutic cargo delivery have been achieved using P-gp-specific antibodies grafted on to very small gold NPs (AuNPs ~4.5 nm) decorated with anticancer drugs on their surface [24]. Last but not least, several recent clinical and preclinical

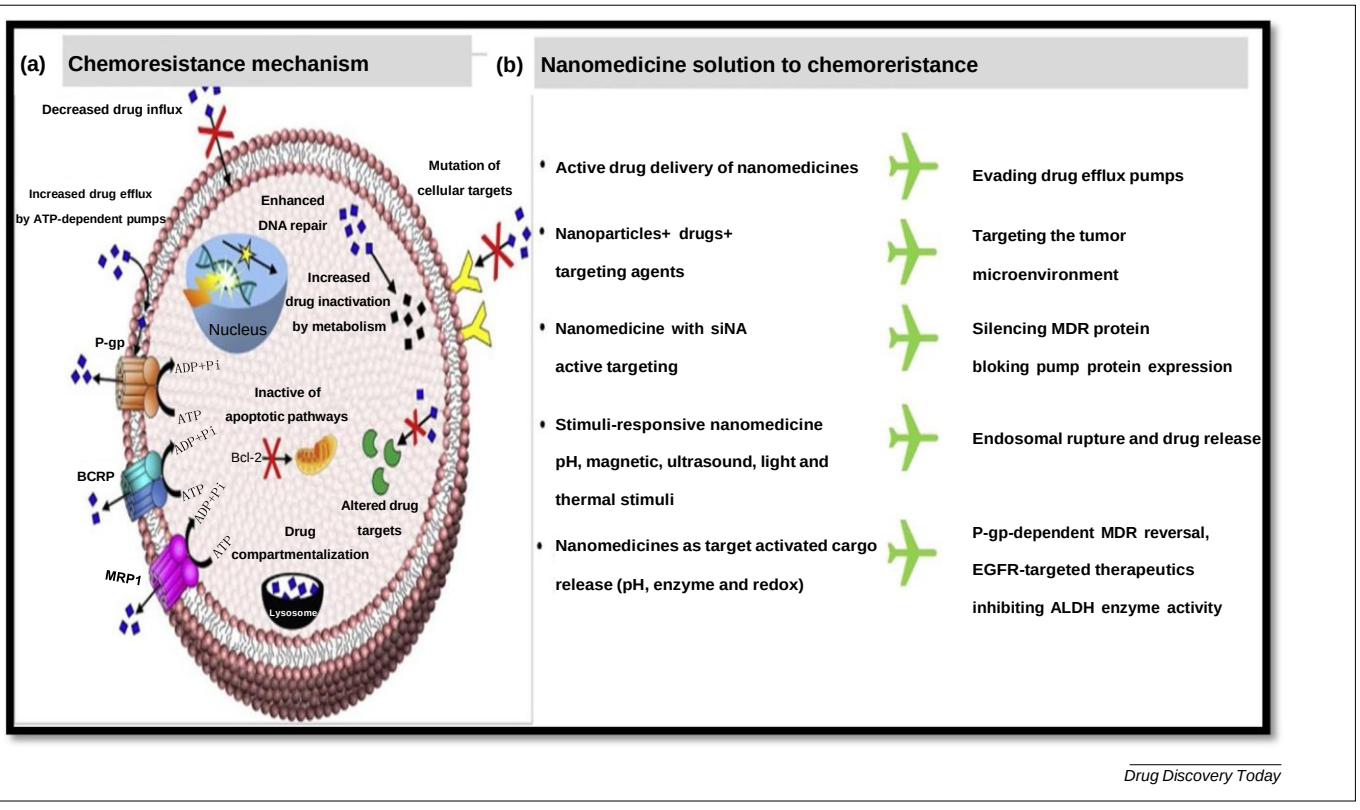

FIGURE 1

Hallmarks of chemoresistance mechanisms of tumor cells and nanomedicine-driven therapeutics for chemoresistant tumors. (a) Chemoresistance is acquired through increased expression of drug efflux transporters, tumor microenvironment (TME) regulation, increased epigenetic miRNA regulation, drug–target alterations, failure to undergo apoptotic signaling pathways, enhanced aldehyde dehydrogenase (ALDH) activity, enhanced DNA repair mechanisms, among other (b) Nanomedicine driven-molecular targeting solutions to tackle the chemoresistance in clinical settings [4]. Abbreviations: EGFR, epidermal growth factor receptor; MDR, multidrug resistance; P-gp, P-glycoprotein; siRNA, small interfering RNA.

BOX 2

Nanomedicine-driven molecular targeting

'Nanomedicine-driven molecular targeting using DNA aptamers, anti-MDR siRNAs, antibodies, folic acid, transferrin, and cell membrane-penetrating peptides ensures efficient delivery of chemotherapeutic cargoes into chemoresistant TME and deep into cells. These nanoplatforms bypass drug efflux pumps and further inhibit P-gp activity.

studies demonstrated that nanomedicine-driven molecular targeting and localized anticancer drug delivery can act in synergy toward P-gp suppression and result in efficient anticancer therapy against chemoresistant tumors [20,25–31].

Receptor-based nanomedicine targeting strategies

Active targeting of cancer cells by nanomedicines relies on strategies based on NP surface decoration with ligands expected to elicit specific drug homing, increase retention at the target tissue/organ, and maximize uptake by the target cells [32]. These ligands, in turn, are chosen/designed to specifically bind to transmembrane receptors that are overexpressed/clustered on the cancer cell surface, thereby limiting indiscriminate destruction of normal cells, general toxicity of conventional chemotherapeutic drugs, and possibly the development of MDR. However, actively targeted NPs must first reach their target to fully exploit their anticancer potential and, for this, they must also have efficient passive targeting properties. Therefore active cellular targeting has been developed as a complementary strategy to EPR-based NPs for improving their tumor localization by increasing their targeting efficiency and increasing retention at the target site [33]. Unfortunately, several studies concurred that even though NPs decorated with targeting moieties showed increased target cell internalization, this did not result in increased tumor localization compared with nontargeted NPs [34], especially when the NP diameter was >50 nm [34].Targeting strategies have been exploited to effectively and selectively deliver any possible therapeutic agent (i.e., from small drugs to proteins and nucleic acids) to malignant cancer cells, with the further purpose of protecting these cargoes from degradation by enzymes and to facilitate their passage through cellular membranes. Yet, NPs decorated with targeting ligands must overcome additional obstacles that are paradoxically ingenerated by their interactions with the target cells. One of the most complex is undoubtedly so-called 'endosomal escape'. Depending on the NP-specific endocytosis mechanism (phagocytosis or pinocytosis), NPs and their cargoes are subjected to intracellular trafficking mechanisms, which can have a detrimental effect on their ultimate fate (e.g., lysosome degradation).

Although an arsenal of strategies has been developed to successfully circumvent endosomal entrapment (e.g., NP derivatization with pore-forming peptides and proteins and pH-buffering substances exploiting the so-called 'proton sponge effect'), escaping from the endosome into the cell cytosol, particularly in vivo, remains one of the main challenges in nanomedicine. As an example, by directly detecting colloidal-Au particles conjugated to siRNAs delivered by LNPs, it was estimated that escape of siRNAs from endosomes into the cytosol occurs at very low efficiency (1–2%) and only during a limited window of time when the LNPs reside in a specific compartment sharing early and late endosomal characteristics [35]. This report emphasizes the importance of improving our understanding of the mechanisms of endosomal escape in different cell types to achieve improved cargo delivery efficiency, which in turn could yield better therapeutic outcomes with fewer off-target effects. Furthermore, this could have wide implications in terms of reducing the toxicity and economic concerns of nanomedicines.

The choice of a NP-targeting moiety is typically based on known disease markers [e.g., G-protein-coupled receptors (GPCRs), integrins, folate receptors (FRs), transferrin receptors (TfRs), and epidermal growth factor receptor (EGFR)]. Nonetheless, although these receptors are undoubtedly clinically relevant for targeting by monoclonal antibodies (mAbs), they might not be equally fit for efficient/effective NP uptake and subsequent endosomal escape in those cases where the cargo must be released within the cellular cytoplasm or even in the nucleus. As a consequence, NP targeting can be tailored only on the basis of a given receptor expression level, but also on the ability of the specific receptor to undergo fast and quantitative internalization and, possibly, endosomal escape.

Another aspect of complexity is added by the heterogeneous nature of tumors and the occurrence of cancer/metastasis-supporting stroma (basement membrane, fibroblasts, extracellular matrix, immune cells, and vasculature). These two factors cannot be considered easily, if at all, when producing targeted NPs because most target just one single transmembrane receptor. Accordingly, tumor heterogeneity is usually neglected even though this might ultimately promote the selection and survival of resistant clones. In the end, as for nontargeted cancer NPs, after the achievement of partial/complete response upon delivery cancer targeted nanomedicines, resistant tumor relapse is still often observed even with these more complex NPs.

Targeting persistent cancer cells

Cancer chemoresistance involves multiple mechanisms, among which the presence of persistent cancer cells (PCCs) has an important role in intrinsic drug resistance, leading to tumor relapse. The dense reservoir of PCCs is responsible for the emergence of drug resistance and, thus, targeting these cells presents a therapeutic opportunity to impede tumor relapse [36]. For instance, nanomedicine-driven molecular localization of reactants, such as Fe^{2+}, Fe^{3+}, and H_2O_2, can accelerate the Fenton reaction. This further initiates an iron-dependent cell death pathway of PCCs that differs from apoptosis at the morphological, biochemical, and genetic levels [37,38]. This new-generation therapeutic approach is termed 'ferroptosis' and can be advantageous at the clinical stage because it offers a solution to the inevitable biocarriers of traditional apoptotic therapeutic means [39]. Molecular targeting of novel nanomedicines shows the potential to trigger ferroptosis for PCCs, particularly for eradicating aggressive chemoresistant tumor cells [40]. Recently, there has been significant effort to design and develop nanomedicine products and targeting platforms for ferroptosis induction. Fe_3O_4/Gd_2O_3 hybrid magnetic NPs loaded with cisplatin and conjugated with lactoferrin (LF) and RGD dimer (RGD2) molecules, were exploited as a ferroptosis therapy module for aggressive orthotopic brain tumors [38]. In another study, novel amphiphilic copolymer nanomicelles were developed using arachidonic acid and the potent ferroptotic inducer RSL3 to target

glutathione peroxidase 4 (GPX4) of PCCs [41]. This nanoassembly targets the TME of chemoresistant human ovarian adenocarcinoma cells and further initiates rapid anticancer cargo release upon free radical triggering. Other studies also reported novel strategies based on nanomedicine-driven molecular targeting coupled with magnetic resonance imaging (MRI) to overcome cancer chemoresistance via tailored ferroptotic pathways and the concomitant monitoring of therapy progress [42–47]. These undoubtedly open new avenues for managing chemoresistant tumors in advanced clinical settings.

Targeting tumor microenvironment

The TME presents challenges to the passive diffusion of chemotherapeutics because it constitutes an indefinite obstacle to drug tumor penetration/accumulation and effective anticancer drug delivery. The TME comprises many barriers to the transport of drugs, such as their circulation, accumulation, penetration, internalization, and release; hence, the TME contributes significantly to chemoresistance in solid tumors [48]. New preclinical evidence suggests that the mechanism of anticancer drug entry into solid TMEs driven by nanomedicine coupled with a molecular targeting process can enhance the efficiency of chemotherapeutics delivery and overcome the barrier of chemoresistance [49,50]. Inspired by the enzyme-triggered transcytosis pathway, nanomedicine-driven and active transport of chemotherapeutics across the capillary wall into solid TMEs was recently proposed to overcome chemoresistance. Specifically, Zhou *et al*. presented a g-glutamyl transpeptidase-responsive camptothecin–polymer nanoconjugate that actively infiltrates throughout the TME by transcytosis [48]. This novel strategy resulted into the eradication of large established tumors (tumor volume ~500 mm^3) and significantly extended the survival rate of mice orthotopically implanted with patient-isolated pancreatic tumors. In another investigation, Wang and coworkers developed a dendrimer-camptothecin (CPT) conjugate that actively penetrated deep into solid pancreatic tumors and facilitated its transendothelial and transcellular transport, thereby augmenting TME accumulation in advanced clinical settings [51]. Collectively, these new-generation molecular nanocarriers all have the potential as nanomedicine-based TME-activated anticancer therapies for chemoresistant tumors.

The TME has a complex dynamic network of both cancerous and noncancerous cells, including immune, vasculature, stroma, and endothelial cells. It is also populated by unique tumor-associated cells that secrete complex growth signaling molecules for tumor survival and growth [52]. Moreover, the TME has distinctive features, such as vascular abnormalities, reduced pH, special metabolic state, high interstitial fluid pressure, and different oxygenation, all hallmarks that differentiate it from surrounding normal cells [53]. Accordingly, exploitation of these unique TME properties in conjunction with the utilization of the molecular targeting ability of nanomedicines could be a useful tool to third-generation dendrimer-based nanocapsule modified with polyethylene glycol (PEG) via a redox-response linkage (mG3) was recently introduced as a functional delivery and therapeutic platform to revert chemoresistance [54]. This nanomedicine platform enabled the water solubility of hydrophobic drugs and increased dose exposure at the tumor site, leading to reversal of developed pancreatic cancer chemoresistance. Similarly, another study reported multifunctional AuNPs grafted with anticancer agents and a DNA aptamer (AS1411) to revert chemoresistance in leukemia [55].

The use of molecular nanoassemblies might be key to unlocking the multilayered shield of the TME (tumor vasculature, stroma, cells, tumor-associated fibroblasts, tumor-associated macrophages, and ECM) and, therefore, achieve precise intracellular chemotherapeutic payload delivery and release to cancer cells [52]. Thus, a new nanotransformer was prepared by self-assembling DOX, tannic acid, and indocyanine green molecules with the ultimate goal to avoid both the nonspecific binding of these NPs to plasma components and their phagocytosis by the reticuloendothelial system (RES) [56]. This molecular nanoassembly rapidly reverted MDR by releasing multiple payloads in the TME. ATP is a prime molecule for regulating tumor progression in the TME, and ATP-dependent drug efflux significantly decreases the localization of therapeutics and chemotherapy efficacy. A minimalist but versatile molecular nanoassembly Fe^{3+}/tannic acid-modified DOX NP ('DFTNP') has also been successfully constructed for TME-responsive drug release to achieve ATP-depletion sensitized chemotherapy and, finally, inhibition of chemoresistant tumor growth [57]. Furthermore, TME-enhanced imaging and photothermal therapy (PTT) for intelligent tumor theranostics was also achieved using the novel molecular nanoassembly strategy outlined in Fig. 2a under multimodal imaging guidance. An alternative approach used tumor-targeted amorphous calcium carbonate (ACC) nanoassemblies with molecular targeting functionality. These were designed to achieve intracellular drug localization and self-regulated release through acidity-triggered degradation of ACC [58]. The resultant nanoassembly was reactivated by the TME and induced tumor cell death via a complementary ferroptosis/apoptosis mechanism (Fig. 2b).

Owing to the special multifunctional features of nanomedicines, NPs with immobilized characteristics can fulfill the diverse demands for in vivo transportation into the TME. Indeed, integration of moieties responsive to TME-related stimuli (e.g., pH, redox, interstitial pressure, and presence of ATP) in NPs is essential for the precise and controlled delivery of therapeutics cargo into TME. A light-responsive nanocomposite was recently fabricated to generate reactive oxygen species (ROS) and trigger TME decomposition (Fig. 2c) [59]. Preclinical anticancer studies demonstrated an effective accumulation and great therapeutic efficacy of this multifunctional nanocomposite in chemoresistant breast tumors, showing the potential of this system as a treatment in advanced clinical settings. Similarly, a novel molecular nanoassembly was developed with dual ferroptosis/apoptosis-inducing capabilities. This organic nanoassembly of a negatively charged polymer [(PEG) and dimethyl maleic acid-grafted polyallylamine (PEG-PAH-DMA)] and a positively charged anticancer prodrug [podophyllotoxin (PPT) conjugated with polyamidoamine (PAMAM) dendrimer with a disulfide linkage (PPT-ss-PAMAM)] along with a molecular targeting moiety on the surface initiated a cascade reaction to achieve TME-triggered drug (PPT) release (Fig. 2d) [60]. Additionally, the multimodal features of nanomedicines can promote internal/external stimuli-responsive effects in the TME, which can increase drug release by a few-fold more than that of conventional drug delivery platforms [61,62].

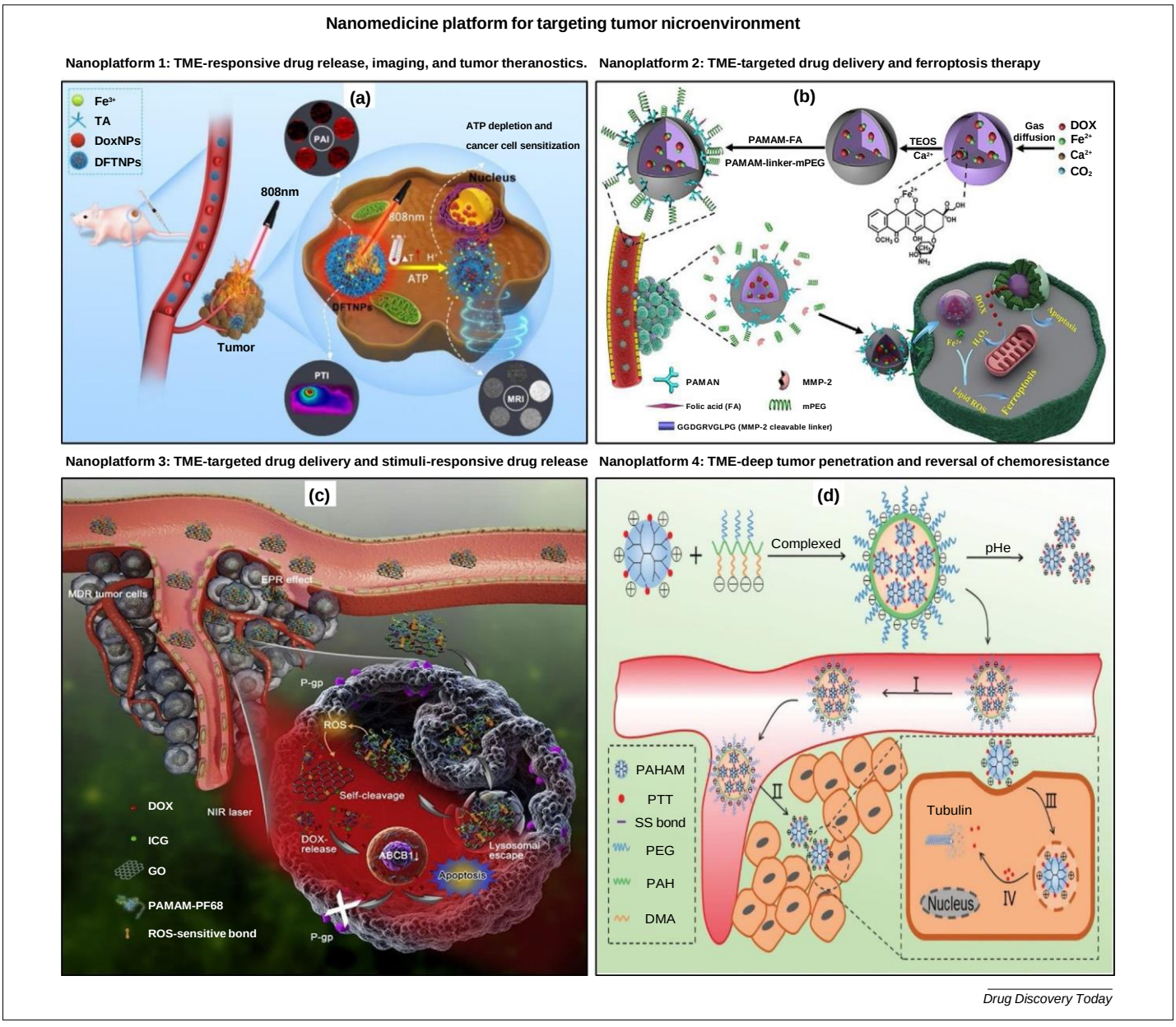

FIGURE 2

Nanomedicine platforms for targeting tumor microenvironment. (a) Nanoplatform 1: molecular nanoassembly as a drug delivery and theranostic platform in the tumor microenvironment (TME) to overcome chemoresistance. This nanoassembly enhances cellular ATP depletion, resulting in simultaneously enhanced cancer cell chemosensitivity, magnetic resonance imaging (MRI) performance, and therapeutic efficacy in preclinical stages [56]. (b) Nanoplatform 2: a TME-activatable Fe-doxorubicin (DOX)-preloaded amorphous $CaCO_3$ nanoformulation triggers ferroptosis in TME and overcomes chemoresistance in clinical settings [57]. (c) Nanoplatform 3: schematic illustration of a photosensitizer containing a reactive oxygen species (ROS)-responsive nanocomposite (GO-PPF68) for overcoming tumor chemoresistance using light-responsive nanotheranostics [58]. (d) Nanoplatform 4: nanomedicine-driven molecular targeting of TME using DMA-NPs that effectively overcome physiological barriers. This nanoformulation further activates a drug delivery cascade dependent on TME-triggered size and charge transformation and suppression of chemoresistant tumors [59].

Targeting tumor hypoxia

Hypoxia is a hallmark of malignancy and a common feature of chemoresistant tumors. It is a biochemical process within a tumor resulting from an imbalance between oxygen supply and consumption in cancer and stromal cells [63]. This further leads to tissue oxygen deficiency because of the aggressive proliferation of cancer cells. Hypoxia activates hypoxia-inducible factor (HIF)-dependent signaling, which initiates metabolic reprogramming, immune suppression, resistance to apoptosis, angiogenesis, metastasis, and invasion of secondary sites [64]. Hypoxia is a function of heterogeneity in oxygen concentration in normal and cancerous tissues and is typically categorized as physiological hypoxia (≤2% O_2, 15 mmHg), pathological hypoxia (≤1% O_2) and radiobiological hypoxia (≤0.4% O_2). The new generation of targeted molecular nanomedicines offers the exploitation of hypoxic TME by (i) enhancing oxygen levels within the tumor; (ii) sensitizing hypoxia using functional NPs; (iii) suppressing HIF; and (iv) stimuli-responsive nanomedicines that alleviate tumor hyp-

oxia for boosting chemotherapy, immunotherapy, and other non-conventional therapies, either individually or in combination.

In the design of hypoxia-responsive nanomedicines, bioinspired cell membrane-coated mesoporous molecular nanocarriers have received considerable attention because of their ability to sequentially delivery two active molecules and overcome niche-mediated chemoresistance by suppressing the hypoxia state in current leukemia treatments (Fig. 3) [65]. Development of an MDR inhibitor that mitigates the hypoxic environment is a further, essential requirement for chemoresistance tumor management. Accordingly, biocompatible and biodegradable catalase-conjugated iron oxide NPs (Cat-IONPs) capable of converting ROS to molecular oxygen to supply an oxygen source for the hypoxic TME and inhibit MDR were recently proposed [66].

Given that the level of oxygen is drastically lower in tumor hypoxic conditions compared with healthy surroundings, a suitable approach to regulate the deep hypoxic tumor is to increase the intratumoral oxygen and anticancer payload. In this context, a versatile nanoscale magnetosome with a molecular targeting ability for the systematic delivery of HIF-1 siRNA in deep hypoxic tumors was developed [67]. This sophisticated nanoplatform comprised magnetic nanoclusters loaded with siRNA molecules and camouflaged with a macrophage-tumor chimeric cell membrane, required to decoy macrophages during tumor targeting. Next, these assemblies were further decorated with dibenzocyclooctyne (DBCO)-modified hyaluronidase (HAase, H) to promote intratumoral penetration via hyaluronic acid (HA) degradation in the tumor ECM. Such packed nanoscale magnetosomes showed several advantages in the programmed delivery of HIF-1 siRNA, including prolonged circulation time, MRI guidance, magnetic tumor accumulation, hypoxic site penetration, homotypic tumor targeting, and cytoplasm trafficking of chemoresistant tumors.

Meanwhile, to enhance tumor cell-penetrating ability and O_2 transport to deep tumor tissues, and reduce RES recognition, a new O_2 self-supplemented nanoplatform was recently developed [68]. This featured cell-penetrating peptides to target hypoxic TME and the ability to boost photocytoxicity under light stimulation.

Hypoxic regions are located deep in tumor tissues, ~100–180 μm from a functional blood vessel [69], and an hypoxic state occurs once the tumor volume increases beyond a certain value (≈0.1 mm^3). Thus, most hypoxic region in solid tumors cannot be reached via conventional, passive diffusion drug-targeting

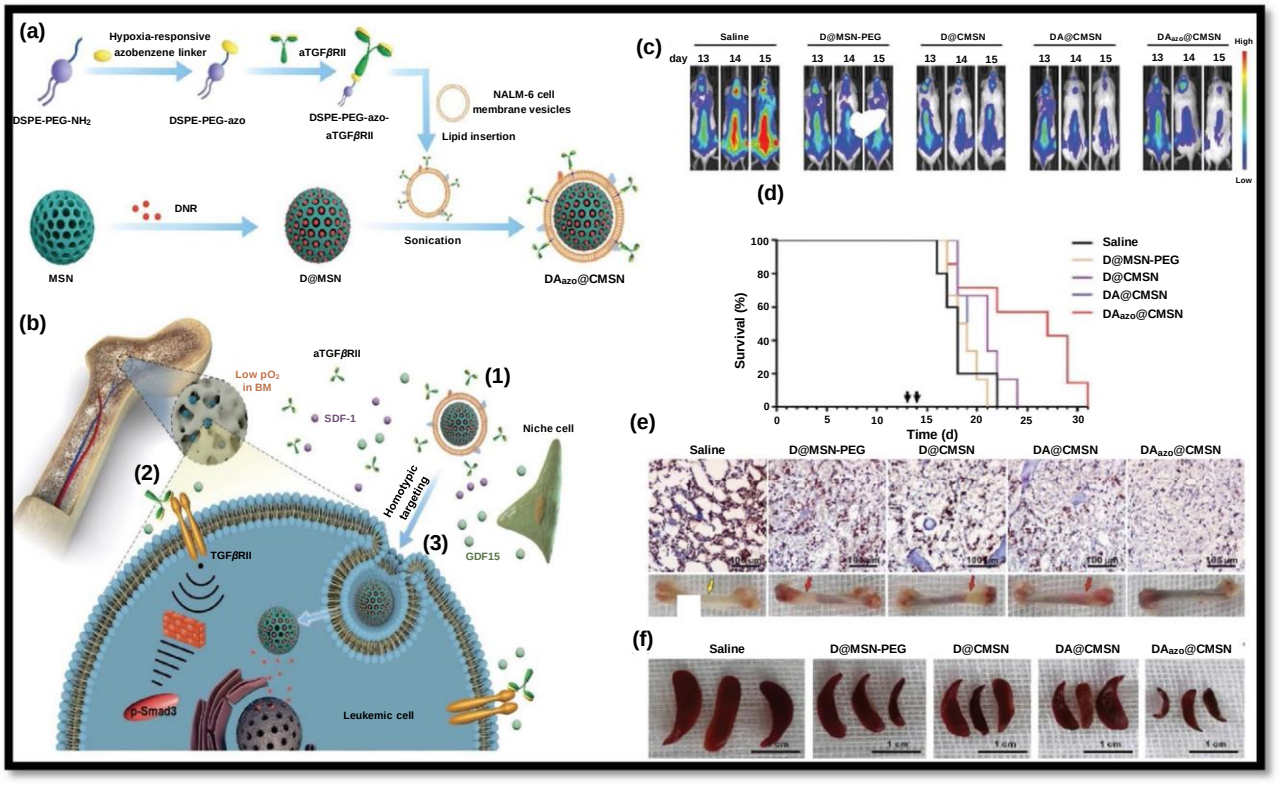

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FIGURE 3

Schematic illustration of (a) nanoengineering and (b) the proposed mechanism to overcome BM TI-niche (therapy induced niche (TI-niche) within the bone marrow microenvironment or niche)-mediated chemoresistance. 1) Through CXCR4-SDF-1 interaction, DAazo@CMSN home into the BM and then TI-niche, where aTGFbRII is released from the nanoparticle surface through the cleavage of hypoxia-responsive azobenzene linker under low pO2 BM microenvironment. 2) aTGFbRII blocks the TGFbRII signaling (p-Smad3) stimulated by GDF15, which is matured by TI-niche cells, to overcome chemoresistance. 3) The remaining D@CMSN were taken up by leukemic cells through homotypic targeting for intracellular DNR delivery and chemotherapy (c) Bioluminescent images of mice bearing induced chemoresistant tumors treated with various targeted nanocarriers and (d) the relevant survival curves (arrow heads indicate two nanoparticle (NP) injections, on Days 13 and 14). (e) Mouse femurs immunohistochemically stained to identify invaded leukemic cells in bone marrow. (f) Morphology of spleens from mice treated with different approaches [65].

approaches. However, nanomedicine-driven molecular targeting approaches can deliver therapeutic payloads deep within solid tumor tissues, thereby providing a window of opportunity for hypoxic TME drug delivery and chemoresistance bypass. Different nanomedicine-driven molecular targeting perspectives have been presented. A size-shrinkable gelatin-based molecular nanovehicle that best balances the performance of blood circulation and tumor penetration was developed to overcome hypoxia by multistage nanocarrier delivery [70]. Poor tumor penetration and hypoxia-induced chemotherapy resistance were avoided by using a simple copolymer poly-[PEG methyl ether meth-acrylate-co-(2-methyl-propenoic acid-glycerol-cinnamaldehyde)] (PgEMC) loaded with DOX. The optimized, small-diameter (60 nm) nanoobjects actively delivered their DOX-cinnamylaldehyde payload in deep tumors and initiated self-inducing ROS by the intracellular pH-sensitive release of anticancer moieties into the breast tumor [71]. Spatio-temporal delivery of therapeutic cargoes was also achieved by designing molecular shell-stacked NPS (SNPs). Such nanoassemblies could co-encapsulate multiple therapeutic agents, and proved to be highly beneficial in the reversal of hypoxia-induced drug resistance of patient-derived colon tumors [72]. Next-generation molecular nanomedicines with an active targeting ability could also constitute an important strategy to overcome tumor heterogeneity and, and such, could be used to design diagnostic and therapeutic strategies for a range of chemoresistant solid tumors [73]. As discussed for hypoxia, molecular nanomedicine active drug delivery strategies have many advantages over conventional passive delivery approaches. Indeed, in recent years, nanomedicine-driven molecular targeting has seen significant advances in the development of hypoxia-active therapeutics, which, in turn, opened new avenues for more effective and efficient applications of NPs against chemoresistant tumors [74–76].

Right place, right dose, and right time ('3R') drug delivery and theranostics platforms

Nanomedicines have the potential to direct chemotherapeutic cargoes to deep solid TMEs and to improve therapeutic activity. Indeed, nanomedicines could ensure that chemotherapeutic treatments act locally and not systemically, thus, potentiating their anticancer efficacy while reducing damage to healthy surroundings. However, recent setbacks, including localized delivery efficiency claims of <1%, have stimulated discussions about the usefulness of nanomedicines for cancer treatment [77,78]. Recent discussions outline that the localized drug delivery field is blocked because of immature preclinical animal studies and the lack of basic information on NP PK in the human body. To maximize tumor drug localization and improve patient lives, the right place, right dose, and right time' 3R' drug delivery principle is proposed [79]. The '3R' drug delivery principle posits to reach the right place (i.e., TME) of chemoresistant tumors and efficiently trigger the drug at the right time and with the right dose (Fig. 4a,b) [4]. Nanotheranostics (nanomedicine + therapy + diagnostics)-based approaches together with external or internal stimuli (responsive/triggered/remotely controlled) facilitate drug delivery to the right place and achieve relatively efficient therapeutic activities at a fixed dose [80–82].

Given that a chemotherapeutic delivery efficiency of systemic platforms of <1% might not be very effective against chemoresis-

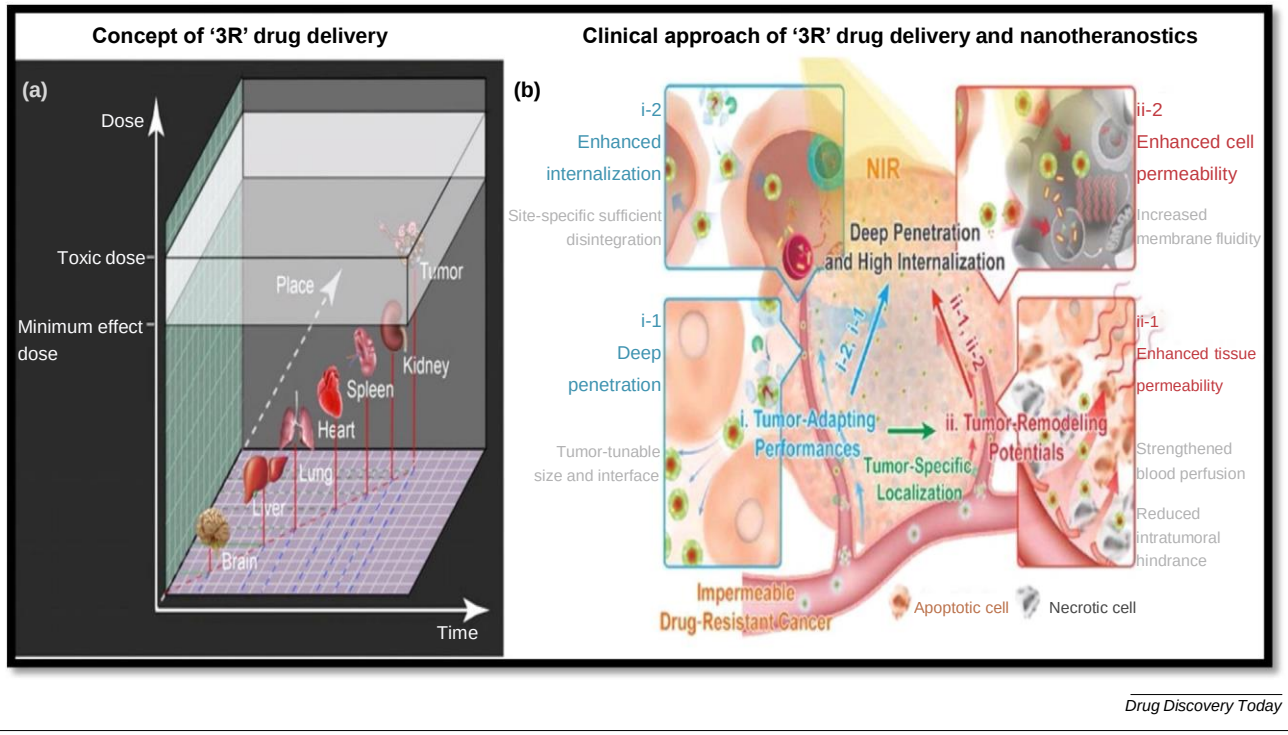

FIGURE 4

Concept and clinical approach of '3R' drug delivery and nanotheranostics. (a) A schematic illustration of pharmacotherapy that is characterized by the '3R' drug delivery principle [4]. (b) Synergy of '3R' drug delivery and nanotheranostics for deep tumor penetration and high internalization of chemoresistant tumors at advanced clinical stages (i-2: Enhanced internalization; ii-2: Enhanced cell permeability; i-1:Deep penetration; ii-1: Enhanced tissue permeability) [77].

TABLE 1

Recent developments in '3R' drug delivery and nanotheranostics treatments in combination with chemotherapy to overcome tumor chemoresistance

Treatment method	Nanomedicine and drug	Tumor model	Drug resistance mechanism	Remarks	Refs
Chemotherapy, gene therapy, and PTT	PPAuNCs with DOX	HepG2/ADR cells	HA to condense miRNA-21 inhibitor	Enhanced therapeutic effects because of combination of chemotherapy, gene therapy, and PTT that efficiently reversed MDR by upregulating expression of PTEN	[97]
Hyperthermia	Polyglycerol-covered nanographene [nG /polyglycerolamine (hPG_{NH2})] with DOX	HeLa-R cells	Triphenylphosphonium (TPP), a mitochondrial-targeting ligand with pH-triggered surface charge conversion	Efficiently and selectively delivered drug to nucleus of cells leading, to high anticancer effect	[94]
	Bimetallic gadolinium super-paramagnetic iron oxide (Gd-SPION) with redox probe	MDA-MB-231 cells	Ascorbic acid (AA) to generate POS	Reduced systemic toxicological dose of drugs and adverse effects of hyperthermia for effective destruction of resistant cancer cells	[96]
PDT	PLGA-lipid hybrid NPs (PLHNPs) with etoposide and PTX	A549 Human lung adenocarcinoma	Delivery ability of PLHNPs	pTHPP-loaded PLHNPs/PDT overcame metastasis-associated and drug-selected resistance in lung cancer	[86]
	Photoresponsive nanocluster (NC) comprising polydopamine (PDA) NPs	MCF-7/ADR cells	D-α-tocopheryl PEG 1000 succinate (TPGS) drug efflux inhibitor	Complementary interactions among PTT/ PDT/CT modalities enhanced efficiency of combined therapy for MDR tumors	[87]
	BSA-HA-carbon dot-based nanoplatform with metformin (Met) and DOX	S180 ascitic tumor cells	Met, an anti-hyperglycemic agent, improves tumor oxygenation to overcome hypoxia	Promising as traceable imaging-guided system for efficient chemotherapy though improved hypoxic TME	[88]
	Aerosol-OT ™ (AOT) –alginate nanocarrier loaded with ME and DOX	JC cells	MB acts as efflux inhibitor for DOX	PDT combined with DOX using MB improved DOX accumulation in tumor; simultaneous localizing of both drugs might result in optimum efficacy	[89]
	Polymeric prodrug micelle with PTX	SKOV-3 and SKOV-3/ MDR cells	P-gp inhibitor tariquidar (TQR) to reduce efflux	Preferential accumulation in tumor tissue with enhanced inhibition of tumor growth	[85]
PTT + chemotherapy	Au nanorod (AuNR)-loaded HA nanogels with DOX	MCF-7 ADR cells	Cystamine (Cys) to remain stable under physiological conditions and release DOX rapidly to mimic intracellular glutathione condition	Hyperthermia effect of PTT changed fluidity and permeability of cell membrane, which increased accumulation and retention of DOX	[95]
PTT	AuNP-coated porous silicon microparticle (AuPSM) with docetaxel micelles (mDTXs)	MDA-MB-231 and SUM159 TNBC cells and mice with SUM159 and 4T1 orthotopic tumors	Chemo-mild hyperthermia combination inhibits CSC activity	DTX contributed to CSC killing by suppressing HSP27 expression, minimized thermal injury to surrounding breast tissue and dermis	[98]
UST	PLGA-based alkaline nanorobots (AN-DSP) containing DOX, sodium carbonate and perfluorocarbon (PFC)	MHCC-LM3	Neutralization of acidic TME by neutralizing lactic acidosis	Enhanced accumulation via enhanced EPR effect, recovering lactic acidosis-mediated drug resistance and systemic disruption of acidic TME by responding to external UST	[91]
	Liposome encapsulating QUE and Adriamcin/DOX (AMD/DOX)	HL-6/ADR and MCF7/ ADR	Quercetin (QUE) as P-gp inhibitor	AMD/QUE/liposome efficiently reversed MDR and lowered cardiac toxicity of AMD	[92]
	6-Shogaol (6S)-loaded phase transition nanobubbles conjugated with Mucin 16 antibody (6S@NB-MUC16) with PTX	OVCAR3 AND A2780 Cells	Inhibition of Toll-like receptor myeloid differentiation factor 2/ myeloid differentiation factor 88 (TLR-MD2/ MyD88)	Enhanced sensitivity of epithelial ovarian cancer (EOC) to PTX and reduced toxicity against critical organs	[93]
	Luteinizing hormone releasing hormone (LHRH) receptor and elastin-like polypeptide drug (DOX) nanoconjugate	MCF7/ADR	Active targeting of overexpressed LHRH on tumor cells	Prolonged circulation half-life, reduced nonspecific cellular uptake, enhanced tumor cell uptake and controllable drug release	[90]

tant tumors, a' 3R' drug delivery approach in combination with nanotheranostics has become a new paradigm for chemoresistant cancer therapy, leading to the development of increasingly complex regimens. In addition, several nanotheranostics approaches to image-guided drug delivery and therapy [83], including light-active molecular nanovesicles for chemo/starvation/chemodynamic trimodal combination therapy [84], or higher doses of chemotherapy with stimuli-responsive nanodrug self-assembled from amphiphilic drug–inhibitor conjugates [23], resulted in improved success of the '3R' drug delivery approach in combination with nanotheranostics by preventing early regrowth of chemoresistance.

Here, we present a framework for a '3R' drug delivery approach in combination with nanotheranostics for chemoresistant cancer management based on recent progress in nanomedicine development (Table 1) [85–98]. Nanomedicines describe standard and emerging interventions that overcome earlier drug delivery issues, and lead cancer management by expanding technological and pharmacological advances to prevent, delay, or revert resistance to therapy [77,99,100]. By responding to external stimuli, such as magnetic field, light, ultrasound and thermal, as well as internal stimuli, such as pH, redox potential, hypoxia and enzymes, nanocargoes improve drug accumulation at the target site by overcoming MDR [101,102]. They also provide alternative approaches for cancer treatment in addition to conventional chemotherapy [e.g., hyperthermia therapy, photodynamic therapy (PDT) and ultrasound therapy (UST)] to overcome drug resistance in cancers. The combination of such therapies could result in synergistic therapies that could improve the intracellular delivery of drugs by decreasing the sensitivity of tumors to those drugs [103].

The '3R' drug delivery approach is well suited to achieving timed-release combination treatments. Indeed, the sequential release of the right amount of chemotherapeutic cargoes at the right place, such as TME, has been actively achieved using nanomedicines. New-generation molecular nanomedicine delivery platforms followed by chemotherapy maximize therapeutic effects in different types of chemoresistant tumor (Box 3). Even more importantly, this '3R' drug delivery strategy is as effective as simultaneous combinatorial theranostics without any evidence of adverse effects associated with the latter. Molecular nanomedicines have a tendency to localize in tumors, which achieves the first principle of '3R' drug delivery strategy (i.e., drugs delivered to the right place). The nanomedicine in combination co-encapsulation drugs and molecules ensures the delivery of therapeutics simultaneously at a fixed dose combination, thus achieving the second principle of the '3R' drug delivery strategy (i.e., right dose). The remote activation of nanomedicines using light, magnetic, ultrasound, or magneto-electric energy can achieve the third principle of the '3R' drug delivery strategy (i.e., right time). Thus, the '3R' drug delivery strategy can significantly improve efficacy and reduce adverse effects, rendering it an effective therapeutic strategy for patients.

A brief market and late-stage clinical trials survey of anticancer nanomedicines

To date, nearly 50 anticancer nanomedicines have received approval from the US Food and Drug Administration (FDA) [104] and/or the European Medicine Agency (EMA) and, according to the National Institute of Health (NIH), 243 clinical trials (CTs) involving nanomedicines in cancer theranostics are ongoing [105]. Anticancer chemotherapeutics based on liposomes as nanotechnology platforms were the first class of nanomedicines to reach the marker stage, with Doxil® (also marketed as Caelyx™), a pegylated liposome/DOX hydrochloride formulation originally approved in 1995 for the treatment of Kaposi's sarcoma, ovarian cancer, multiple myeloma, and metastatic breast cancer, and DaunoXome® (NeXstar Pharmaceuticals), comprising heat-activated liposomes loaded with daunorubicin, active against AIDS-related Kaposi's sarcoma and approved just 1 year after Doxil®, well-known examples. Drug encapsulation in these spherical vesicles effectively resulted in improved PK/pharmacodynamics (PD) characteristics of their chemotherapeutic cargos, in many cases accompanied by reduced adverse effects; unfortunately, however, no liposome-based nanomedicine was successful in increasing the overall survival (OS) of patients with cancer compared with the parent drug alone [106]. For example, the results from a Phase III CT of Vyxeos (aka CPX-351), a recent nanomedicine based on a cytarabine–daunorubicin liposomal formulation to treat adults with newly diagnosed therapy-related acute myeloid leukemia (t-AML) or AML with myelodysplasia-related changes (AML-MRC), showed a median OS of 9.6 months versus 5.9 months compared with cytarabine + daunorubicin administered according to the standard-of-care regimen [107].

Abraxane® (Celgene), albumin-bound PTX NPs (Nab-paclitaxel), was approved in 2005 for metastatic breast cancer, in 2012 for first-line treatment of advanced nonsmall cell lung cancer, and in 2013 for late-stage pancreatic cancer. Moreover, in 2019, the FDA also granted accelerated approval for Genentech's Tecentriq in combination with Abraxane® for patients with PD-L1-positive, metastatic triple-negative breast cancer. Encapsulation of taxol, a drug characterized by high general toxicity and water insolubility, into the *Nab* platform avoided the addition of further toxic excipients, ultimately resulting in patient compliance. This, in turn, allowed for the quicker administration of Abraxane® at higher doses, thereby achieving a higher maximum drug concentration in serum (C_{max}) and higher plasma area under the curve (AUC) [108]. However, Abraxane® showed controversial results in terms of dosage and administration regimens. On the one hand, a protocol based on one administration of the nanomedicine every 3 weeks to patients with breast cancer revealed a superior response rate and time to progression with respect to conventional PTX [109]; on the other hand, an alternative regimen based on weekly Abraxane® delivery did not yield similar results in terms of OS and progression-free survival (PFS), but also reported increased toxicity [110]. Nanomedicines based on self-assembled polymeric micelles or polymeric NPs constitute a relatively new class of cancer nanotherapeutics [111,112]. Although conceptually ideal for rational design, synthesis, and characterization, the clinical outcomes of these nanoplatforms (e.g., Genexol-PM®, NK105, CRLX101, and BIND-104) available show conflicting results [113]. For instance, ifforGenexol-PM® improved solubility and efficacy and reduced toxicity and hypersensitivity compared with Taxol [114], but had the limitation that the anticancer cargo is rapidly released from the nanomicelles after intravenous injection, is widely distributed, and is quickly eliminated by hepatic metabolism and bile excretion [115]. For NK105, a micellar PTX

BOX 3

Nanomedicine-driven molecular targeting: development, clinical advancements, and limitations

Nanomedicine-driven molecular targeting

- Characteristics of nanomedicine in chemoresistance therapy:
- Nanoassemblies are ordered and of a uniform size
- NP surface functionality allows multiple chemotherapeutic and targeting cargo payloads
- Multifunctionality for selective intracellular accumulation in cancer cells using both passive and active targeting
- NPs loaded with a drug cargo can facilitate enhanced target-activated drug release mechanisms, thereby minimizing systemic toxicity
- Prolonged circulation properties
- Ability to accumulate in tumors via EPR effect
- Responsive to internal and or external stimuli for remotely activated theranostics
- Nanomedicine modulates the therapeutic index (e.g., drug selectivity, tumor characteristics, target selectivity, and resistance liabilities)

Nanomedicine: advancing the chemoresistance treatment strategy

- Downregulates pump resistance-associated proteins
- Enhances drug delivery efficiency and inhibits drug efflux
- Sequential release promotes drug accumulation in TME
- High penetrate into tumor interstitial fluid of TME with high pressure
- Ability to relieve hypoxic conditions in tumors
- High site-specific delivery (tumor) and solubility
- Prolonged drug delivery and circulation time in body
- Reduces dose-limiting adverse effects

Nanomedicine-driven molecular targeting and theranostics

- Current limitations of nanomedicine-driven molecular targeting:
- Problems of reproducibility of in vitro and in vivo anticancer activity in advanced clinical settings
- Limited clinical evidence for delivering a chemotherapeutic agent with a synergistic combination of drugs and molecular targeting moieties (e.g., DNA aptamers, anti-MDR siRNAs, antibodies, folic acid, transferrin, and cell membrane-penetrating peptides)
- Limited access for target-activated release mechanisms in TME in response to target-specific nano-environmental stimuli
- Limited studies on the combination of therapeutics with diagnostic imaging modalities to facilitate the localization of nanomedicines at the right place, with the right dose and at the right time
- Limited feedback mechanism for monitoring the real-time anticancer efficacy of a delivered therapeutic cargo in the deep tumor or TME
- Current clinical studies rely only on induced or lab-designed clinical models
- Limited understanding of tumor heterogeneity is a major hurdle to achieving synergism of molecular targeting and therapy
- Limited understanding of the controllable and reproducible platforms and scalable manufacturing of nanomedicines
- Lack of trust or interdisciplinary knowledge exchange between nanomedicine researchers, clinicians, oncologists, biomedical scientists, and investigators

Key considerations/ways to overcome limitations

- Nanomedicine providing pH/enzyme/redox-responsive therapeutics to overcome complicated physiological pathological barriers
- Nanomedicine-based nonconventional therapies to enhance drug penetration of impermeable tumor tissues and for drug internalization into chemoresistant tumor cells
- Tumor-adapting performances of nanomedicine contribute to improving site-specific magnetic fluid hyperthermia (MFH), PTT, and PDT, which allows intratumoral penetration, cell internalization, and tumor permeability of associated drugs
- Image-guided synergistic therapy of chemotherapy coupled with MFH, PTT, PDT, and computer tomography (CT)-MRI

formulation comprising PEG as the hydrophilic block and modified polyaspartate as the hydrophobic block with the drug incorporated via hydrophobic interactions, the primary endpoint of non-inferiority of PFS relative to the free drug was not met; however, the incidence of associated hypersensitivity and peripheral sensory neuropathy (PSN) profiles were better than that of PTX. In particular, patient-reported outcomes of PSN were significantly favorable for NK105 [116]. BIND-014 [a PEG/poly (lactic-co-glycolic acid) formulation of docetaxel] is generally well tolerated, with predictable and manageable toxicity and a unique PK profile compared with conventional docetaxel. Interestingly, clinical activity of BIND-014 was noted in multiple tumor types [117,118]. Interestingly, however, the results of these nanoformulations in metastatic castration-resistant prostate cancer underscored the fundamental issue of patient selection, that is, the identification of those affected individuals who can really benefit from therapeutic/toxicity advantages brought about by the nanomedicine over standard drug-based chemotherapy [118].

Inorganic NP-based nanomedicines are also new materials currently actively investigated as cancer nanotherapeutics [119]. In this arena, NanoTherm1 (MagForce) was approved by EMA in 2013, but is still under late-stage clinical trials in the USA. It comprises

aminosilane-coated superparamagnetic iron oxide NPs for local ablation in glioblastoma, prostate, and pancreatic cancer (intratumoral) [120]. Within the same class, Density1 (Nanorobotix, approved by EMA in 2019) comprises hafnium oxide NPs. which are effective against locally advanced soft tissue sarcoma [121].

Besides classical anticancer drugs, most NP-based platforms can also delivery new anticancer therapeutics, which include, among others, siRNAs [122], miRNAs [123], and antisense oligonucleotides (ASOs) [124]. Although no nanomedicine belonging to any of these types have been approved for cancer treatment yet, many are under intense preclinical and clinical study. An archetypal representative of these nanosystems, Patisiran/ONPATTRO (a siRNA delivery lipid-based NP aimed at silencing the faulty gene responsible for hereditary transthyretin amyloidosis [125], approved in 2018) deserves special mention as the first RNAi therapy-delivery nanosystem to receive FDA approval [126].

In addition to the delivery of drugs and nucleic acids, nanotechnology is witnessing unprecedented momentum in cancer immunotherapy. Indeed, nanotechnology provides a unique opportunity to face the challenges associated with immunology and vaccine development [127]. In the first instance, NPs constitute ideal model systems to gain a mechanistic understanding of how the immune system operates, thereby allowing for a systematic identification of key factors and their roles in specific immune responses. Next, the polyhedric chemical nature and the multifaceted architectural features of NPs enable new strategies/novel platforms for developing vaccines with high efficacies and effectiveness. In this specific field, the shift towards the use of subunit antigens, which are much safer but less immunogenic than inactivated or live attenuated vaccines, and the recognized need to facilitate global access to vaccines, are stimulating the quest and search for safe and efficient adjuvants and delivery technologies.

TABLE 2

Examples of clinical-stage nanomedicines[a]

Name	Nanoplatform/drug	Cancer type (CT phase)
Liposome NPs		
Lipoplatin™	Pegylated liposomes/cisplatin	Nonsmall cell lung cancer, head and neck cancer, ovarian cancer, gastric cancer (II,III)
EndoTAG-1	Positively charged liposomes/PTX	HER-2-negative breast cancer, locally advanced and/or metastatic pancreatic adenocarcinoma (II)
Lipusu1	Lecithin and cholesterol liposomes/PTX	Nonsmall cell lung cancer, breast cancer, gastric cancer (II,IV)
Halaven1	Liposomes bearing cyclodextrin in internal phase/eribulin mesylate	Locally advanced or metastatic breast cancer, unresectable liposarcoma (I,II)
LipoMIT	Cholesterol and a diacylphosphatidyl choline/mitoxantrone	Relapsed or refractory peripheral T cell and NK/T cell lymphoma, breast cancer (II)
Promitil1	Pegylated liposomes/mitomycin-C	Colorectal carcinoma, solid tumors (I,II)
JVRS-100	Cationic liposomes incorporating a plasmid DNA complex for immune system stimulation	Relapsed or refractory leukemia (I)
MM-302	HER2-targeted antibody/liposomal DOX conjugate	Advanced HER2-positive breast cancer (II,III)
MBP-426	Liposomal oxaliplatin/folinic acid/5-fluorouracil	Second-line gastric, gastroesophageal, or esophageal adenocarcinoma (I,II)
CPX-1	Liposomal irinotecan hydrochloride/floxuridine (1:1)	Advanced colorectal cancer (I,II)
siRNA-EphA2-DOPC	siRNA liposomes for EphA2 knockdown	Advanced or recurrent solid tumors (I)
TKM-080301	Liposomes for siRNA against PLK1	Neuroendocrine tumors and adrenocortical carcinoma (I,II)
Atu027	AtuRNAi liposomal formulation for PNK3 knockdown	Advanced solid tumors, pancreatic cancer (I/II)
SGT-94	Liposomal RB94 plasmid DNA formulation with antitransferrin receptor antibodies	Solid tumors (I)
SGT-53	Liposomal formulation with antitransferrin receptor antibodies encapsulating wild-type p53 sequence	Glioblastoma, solid tumors, pancreatic cancers (I,II)
DCR-MYC	Double-stranded-siRNA liposomal formulation for MYC silencing	Hepatocellular carcinoma, lymphoma, multiple myeloma, solid tumors (I,II)
BP1001	Liposomal growth factor receptor-bound protein-2 antisense oligodeoxynucleotide (L-Grb2 AS) formulation	Recurrent adult acute myeloid leukemia, acute lymphoblastic leukemia, myelodysplastic syndrome, Ph1-positive chronic myeloid leukemia (I,II)
Polymeric NPs		
NC-6004 Nanoplatin	Nanomicelles of polyaminoacid, PEG, and cisplatin	Advanced solid tumors, lung cancer, biliary cancer, bladder cancer, pancreatic cancer (I,II,III)
NC-4016DACH-Platin	Nanomicelles of polyaminoacid, PEG, and oxaliplatin	Advanced solid tumors and lymphomas (II)
CriPEC	Nanomicelles encapsulating docetaxel	Solid tumors, ovarian cancers (I,II)
AZD2811	Nanomicelles encapsulating an Aurora kinase inhibitor	Advanced solid tumors (I,II)
Protein NPs		
ABI-009	Albumin-bound rapamycin	Bladder cancer (I,II)
ABI-011	Albumin-bound thiocolchicine analog	Solid tumors and lymphomas (I)
Inorganic NPs		
AuroLase	PEG-coated silica-AuNPs	Solid primary or metastatic lung tumors (–)
Cornell dots	Silica NPs coated with PEG and decorated with decorated with NIR fluorophore and ^{124}I-radiolabelledcRGDY peptide	Imaging of melanoma and malignant brain tumors (I,II)
Magnablate	Iron NPs	Prostate cancer (0)

[a] Based on Ref. [105].

In particular, the intrinsic weak immunogenicity of subunit antigens requires the presence of an adjuvant in the relevant formulation. In this respect, NPs constitute the perfect platforms to improve tissue penetration, prolongedantigenpresentationandpreferentialuptake by antigen-presenting cells (APCs), in situ sustained antigen/adjuvant release, and improved antigen phagosome escape for cross-presentation [128–132]. A recent success in this challenging field was the NP-assisted delivery of cyclic dinucleotide (CDN) agonists of stimulatorofinterferongenes(STING), aclassofimmunotherapeutic that activate innate immunity to increase tumor immunogenicity [133]. However, the efficacy of CDNs is limited by drug delivery barriers, including poor cellular targeting, rapid clearance, and inefficient transport to the cytosol, where STING is localized. In 2017, Luo *et al*. reported results obtained with STING-activating NP (STING-NP)-rationallydesigned polymersomes inenhanced cytosolic delivery of the endogenous CDN ligand for STING, 2'3'-cyclic guanosine monophosphate-AMP (cGAMP) [134]. Specifically, STING-NPs were able to increase the biological potency of cGAMP, enhance STING signaling in the TME/sentinel lymph nodes, and convert immunosuppressive tumors to immunogenic, tumoricidal microenvironments. This, in turn, resulted in several, related beneficial effects, including(i) enhancedtherapeutic activity of cGAMP; (ii) inhibitionoftumorgrowth;(iii) increasedratesoflong-termsurvival; and (iv) improved response to immune checkpoint blockade and induction of immunological memory that protects against tumor rechallenge. The final validation of STING-NPs in freshly isolated human melanoma tissue opened new avenues for the potential translation of this nanomedicine in clinical immunotherapy.

Finally, nanomedicines hold potential to overcome at least some of the obstacles related to the administration of biologics, including, for example, mAbs, which constitute the mainstay of cancer immunotherapy. These biological drugs can trigger immune responses that lead to the formation of antidrug antibodies (ADAs). Although clinical consequences remain uncertain, ADAs can affect PK, patient safety, and treatment efficacy [135–137]. A promising example of nanomedicine in this field is SVP-R, a biodegradable, poly (lactic-co-glycolic acid) NP-based formulation comprising synthetic vaccine particles (SVP) encapsulating the macrolide antibiotic rapamycin (R), with immunosuppressant and drug-protective activities. Upon administration of SVP-R, the SVP moiety is selectively and preferentially taken up through endocytosis by APCs prevalently located in the spleen and lymph nodes [specifically dendritic cells (DCs)]. Rapamycin is an inhibitor of the mammalian target of rapamycin (mTOR, a protein kinase activated in response to growth, nutrient and energy signals that leads to an increase in protein synthesis, which is required for tumor development), used in the clinical oncologyofrenalcellcarcinoma[138]. However, rapamycininhibits the response to interleukin (IL)-2, and thereby blocks activation and proliferation of T and B cells, inducing immune tolerance. When co-administered with a biological immunogenic drug known to induce the production of ADAs, SVP-R suppresses ADA formation, thus preventing the unwanted neutralizing effects of ADAs, increasing the biological drug efficacy, and permitting sustained therapeutic activity and repeated administration of the biologic. Also, in the presence of a specific target antigen, SVP-R is able to prevent an antigen-specific immune response and induces antigen-specific immune tolerance. Compared with the administration of free rapamycin, SVP-R induces long-lasting immunological tolerance [139,140].

The arsenal of medicines based on nanoplatforms is continuously expanding, and it is a daunting task trying to keep pace with all the new development and translational information available in the literature. Table 2 provides a brief selection of nanomedicines currently at the investigational stage in the arena of chemoresistant anticancer therapeutics (an extensive list of approved and in-trial nanodrugs can be found in Refs. [121,141]).

Concluding remarks and future perspectives

Overcoming chemoresistance to antitumor drugs is a central goal of cancer research and therapeutics, making it possible to treat these diseases more accurately and effectively. Chemoresistance remains a key obstacle to the success of cancer chemotherapeutics. Owing to its multifunctionality, current-generation nanomedicines have the potential to overcome cancer chemoresistance by enhancing the accumulation of chemotherapeutic drugs in tumors, leading to precise and optimal molecular targeting, and prolonged circulation time, as well as their ability to carry combinations of therapeutic payloads.

Nanomedicines have the potential to direct chemotherapeutic cargoes to deep solid TMEs and to improve therapeutic activity, as well as their transport in tissues. The novel drug delivery approach using molecular-targeting moieties improves the biodistribution of the drug, and PK serves as a reference for the development of improved chemoresistance cancer treatments based on nanodrug combinations. Although these nanomedicines promise improved treatment with high efficacy, complex synthetic routes and high costs might limit their practical applications. Such issues can be addressed by building optimal designs using fewer components while still maximizing functionality. Further chemotherapeutics drug administration routes that are non-invasive are needed to decrease any adverse effects. The advantages of nanomedicine combination therapy and drug delivery at the right place, right time, and right dose (3R delivery principle) has emerged as unique treatment modality with minimal adverse effects and high treatment efficacy. Therefore, this is an effective therapeutic strategy for patients in advanced clinical settings to overcome cancer chemoresistance. One of the major reasons for tumor relapse are the small populations of CSCs and their microenvironment, which needs to be effectively tackled by designing nanomedicine-based strategies using chemical agents that target proteins involved in CSC signaling pathways. Thus, further studies related to the design, screening of various targets, and cellular pathways for the effective and complete eradication of CSCs are needed.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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