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Nanomedicine therapeutic approaches to overcome cancer drug resistance☆☆

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ABSTRACT

Nanomedicine is an emerging form of therapy that focuses on alternative drug delivery and improvement of the treatment efficacy while reducing detrimental side effects to normal tissues. Cancer drug resistance is a complicated process that involves multiple mechanisms. Here we discuss the major forms of drug resistance and the new possibilities that nanomedicines offer to overcome these treatment obstacles. Novel nanomedicines that have a high ability for flexible, fast drug design and production based on tumor genetic profiles can be created making drug selection for personal patient treatment much more intensive and effective. This review aims to demonstrate the advantage of the young medical science field, nanomedicine, for overcoming cancer drug resistance. With the advanced design and alternative mechanisms of drug delivery known for different nanodrugs including liposomes, polymer conjugates, micelles, dendrimers, carbon-based, and metallic nanoparticles, overcoming various forms of multi-drug resistance looks promising and opens new horizons for cancer treatment.

Contents

Abbreviations: AML, acute myeloid leukemia; CAM-DR, cell adhesion-mediated drug resistance; CCP, charge-conversion polymer; CLL, chronic lymphocytic leukemia; CNS, central nervous system; CSC, cancer stem cell; EGFR, epidermal growth factor receptor; EPR, enhanced permeability and retention; HIF-1, hypoxia-inducible factor 1; IL-2, interleukin-2; LLL, H_2N-Leu-Leu-Leu-OH; IGF-1R, insulin-like growth factor 1 receptor; mAb, monoclonal antibody; MDR, multidrug resistance; MRP, multidrug-resistance-associated protein; NF-κB, nuclear factor κB; NSCLC, non-small cell lung cancer; PDGFR-β, platelet-derived growth factor receptor-β; PEG, polyethylene glycol; RES, reticuloendothelial system; SDF-1/CXCL12, stromal cell-derived factor 1; siRNA, short interfering RNA; TAT, transactivator of transcription; TfR, transferrin receptor; TG2, tissue transglutaminase; TLR, toll-like receptor; TMZ, temozolomide; TNFα, tumor necrosis factor α.

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1. Introduction

Resistance to antineoplastic chemotherapy is a combined characteristic of the specific drug, the specific tumor, and the specific host whereby the drug is ineffective in controlling the tumor without excessive toxicity.

The problem for the medical oncologist is not simply to find an agent that is cytotoxic but to find one that selectively kills neoplastic cells while preserving the essential host cells and their functions. Were it not for the problem of resistance of human cancer to antineoplastic agents or, conversely, the lack of selectivity of those agents, cancer chemotherapy would have been similar to antibacterial chemotherapy in which complete eradication of infection is regularly observed.

Natural (inherited) or acquired resistance is one of the main problems associated with cancer treatment. Natural resistance refers to the initial unresponsiveness of a tumor to a given drug, and acquired resistance refers to the unresponsiveness that emerges after initial successful treatment.

There are three basic categories of resistance to chemotherapy: kinetic, biochemical, and pharmacologic. Cell kinetics and resistance is a particular problem with many human tumors because certain cells are in a plateau growth phase with a small growth fraction. Strategies to overcome resistance due to cell kinetics include: reduction of the bulk of tumors with surgery or radiotherapy; using combinations to include drugs that affect resting populations (G_0 cells); and scheduling of drugs to prevent phase escape or to synchronize cell populations and increase tumor cell elimination. How cells become resistant biochemically is only partially understood. The major mechanisms of biochemical resistance include the inability of a tumor to convert the drug to its active form, the inactivation of a drug, and the upregulation of the tumor enzymatic repair systems that counteract the tumoricidal action. Cells in this resistance category can decrease drug uptake, increase efflux, change the levels or structure of the intracellular target, reduce intracellular activation, increase inactivation of the drug, or increase the rate of repair of damaged DNA. Another example is multidrug resistance (MDR), also called *pleiotropic drug resistance*, which is a phenomenon whereby treatment with one agent confers resistance not only to that drug and other(s) of its class but also to several other unrelated agents. Pharmaceutical resistance can result from poor tumor blood supply, poor or erratic absorption, increased excretion or catabolism, and drug interactions, which all lead to inadequate blood levels of the drug. One other example of pharmacologic resistance is poor transport of agents into certain body tissues and tumor cells. For instance, tumors of the central nervous system (CNS) or ones that metastasize there should be treated with drugs that achieve effective antitumor concentration in the brain tissue and are also effective against the tumor cell type being treated.

Novel nanomedicines offering flexible and fast drug design and production based on tumor genetic profiles can be created making drug selection for personalized patient treatment much more rational and effective. This review aims to demonstrate the advantages of nanomedicine in overcoming cancer drug resistance.

2. Classes of nanodrugs used to treat cancer and their current clinical status

Nanomedicines are being investigated for their use in anticancer therapies to improve drug delivery, increase the efficacy of treatment, reduce side effects, and overcome drug resistance. The number of studies published under the research topics of "nanomedicine," "nanoscience," and "nanotechnology" has increased exponentially over the past decade with a slight decline in 2012, as shown in Fig. 1. As more nanostructures were discovered and their potentials were better understood, the number of publications increased and reached its peak in 2011. Currently, the knowledge base of nanoparticles is still expanding with an emphasis on safety and efficacy.

2.1. Lipid-based nanoparticles (liposomes)

Liposomes, as shown in Fig. 2A, are lipid based vesicles that have the ability to carry payloads in either an aqueous compartment or embedded in the lipid bilayer. The delivery of these liposomes to cancer cells often relies on passive targeting and is based on the enhanced permeability and retention (EPR) effect, for which a leaky tumor vasculature is necessary [1]. A number of liposomes with the addition of targeting ligands, such as the mAb 2C5 with Doxorubicin (Doxil®) [2] and an anti-HER2 mAb with Paclitaxel [3], are in the preclinical phase, whereas others are already undergoing clinical trials. Advances to liposome design have also been made with the addition of polyethylene glycol (PEG, known as stealth liposomes), which increases circulation time, as well as strategies for a triggered release of the drug once internalized, such as hyperthermia, as is used in ThermoDox®, which is currently in Phase III trials [1,4,5].

2.2. Polymer-based nanoparticles and micelles

Polymeric nanoparticles, as shown in Fig. 2B, can either covalently attach to or encapsulate therapeutic payloads. Biodegradable synthetic and/or natural polymers are used. Through self-assembly after mixing the drug with the polymers, capsules may be formed spontaneously (micelles, Fig. 2C) or by emulsion techniques as nanosized droplets. These nanospheres contain a solid core that is ideal for hydrophobic drugs, are highly stable, have a relatively uniform size, and are capable of controlled drug release. For water-soluble polymers, drugs can be

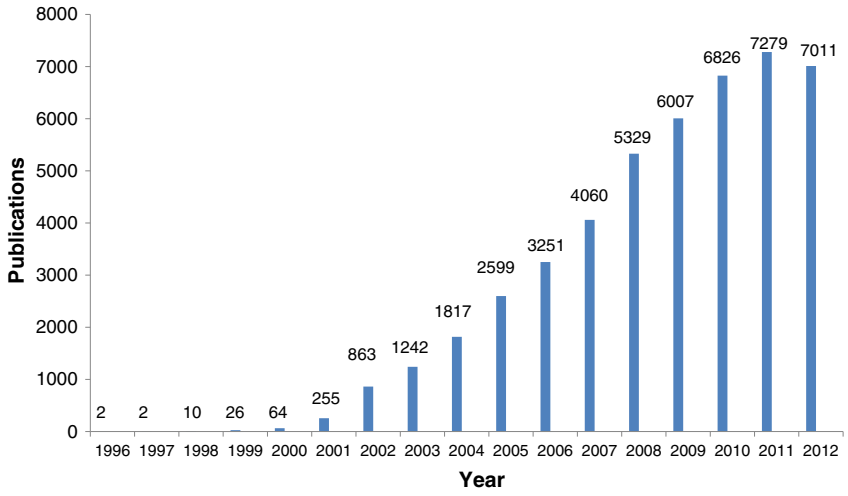

Fig. 1. The number of references under the research topics of "nanomedicine," "nanoscience," and "nanotechnology" from 1996 to 2012. The number of publications peaked in 2011 with 7279 and saw a slight decline in 2012 with 7011 publications.

covalently bound to increase circulation time and limit toxicity to normal tissues [6–9]. Polymers have been refined with the addition of PEG to avoid opsonization and increase circulation time, the use of targeting ligands, and the use of pH-sensitive or hypothermic polymer conjugates. Currently, two polymers, polylactide (PLA) and poly(lactide-*co*-glycolide) (PLGA), are polymeric biodegradable nanoplatforms that are used for synthesis of FDA-approved nanomedicines, whereas many others are undergoing clinical trials [7].

2.3. Dendrimers

Dendrimers are well-defined globular structures of multi-branched polymers that are characterized by a central core, branches of repeating units, and an outer layer of multivalent functional groups, as shown in Fig. 2D. These functional groups can electrostatically interact with charged polar molecules, whereas the hydrophobic inner cavities can encapsulate uncharged, non-polar molecules through a number of interactions. The outer functional groups also allow for controlled delivery of the drug by modifications that only release in a certain pH or when encountered by specific enzymes; targeting molecules, such as the RGD peptide or mAbs are also used. Further, covalent attachment of hydrophobic drugs such as Doxorubicin and Paclitaxel is frequently employed [6,7,10]. Dendrimers, such as poly(glutamic acid)-*b*-poly(phenylalanine) copolymers, can also be self-assembled into micelles to deliver drugs in their core. Multiple clinical trials are ongoing using amphiphilic diblock copolymer forming micelles to deliver Paclitaxel to treat breast, non-small cell lung cancer, and advanced pancreatic cancer [11].

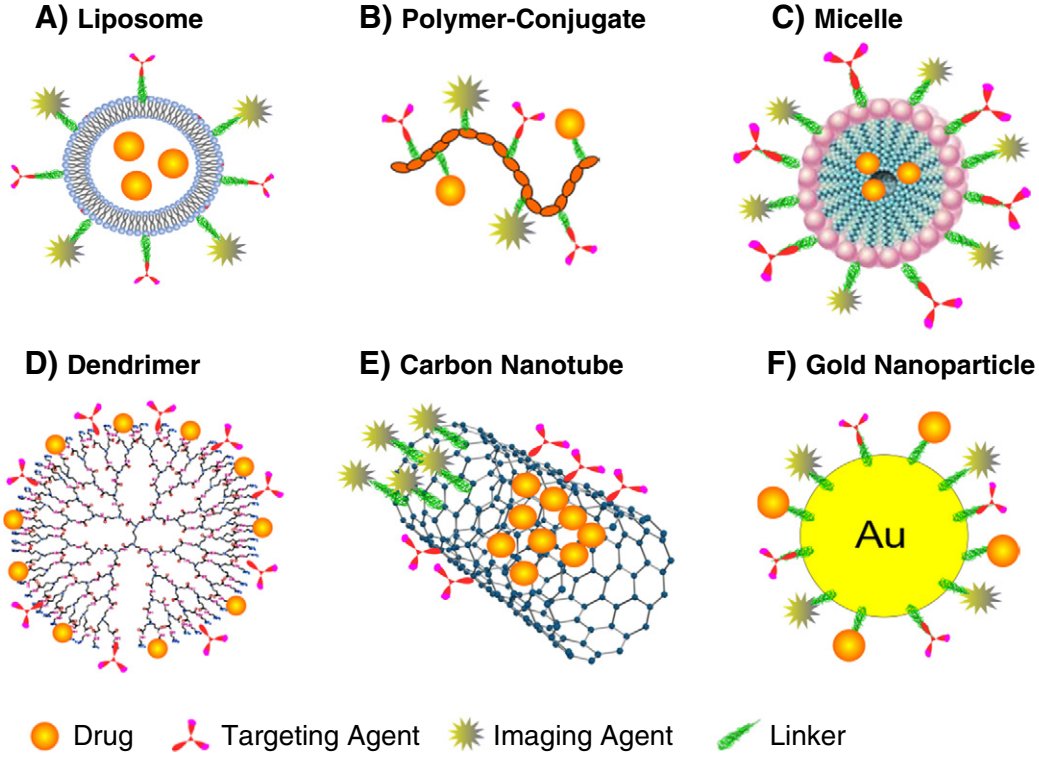

Fig. 2. An illustrative representation of different classes of third-generation multiple functional nanodrugs and their potential moieties for targeting, PEGylated for resistance and with imaging moieties.

2.4. Carbon-based nanoparticles

Carbon nanotubes have the ability to enter cells using "needle-like penetration" and deliver molecules into the cytoplasm. These nanoparticles are equipped with a large surface area providing for a number of attachment sites for potential targeting ligands, as well as an internal cavity that can contain either therapeutic or diagnostic agents, as shown in Fig. 2E. These carbon nanotubes also have electrical and thermal conductivity, which may prove to be useful in future cancer therapy applications such as thermal ablations. The length and diameter of these nanotubes can be crucial for avoiding an inflammogenic effect, making smaller and thicker nanotubes more desirable and a focus on biodegradability necessary. Current approaches to nanotubes include the incorporation of drugs such as Doxorubicin and Paclitaxel, nucleic acids including antisense oligonucleotides and short interfering RNAs (siRNAs), [12] and the use of nanotubes as contrast agents for imaging. To our knowledge, no clinical trials have begun using carbon nanotubes for the treatment or diagnosis of cancer, mainly because of toxicity concerns and their similarity to asbestos fibers [7].

2.5. Metallic and magnetic nanoparticles

Gold nanoparticles, as shown in Fig. 2F, can be used to deliver small molecules such as proteins, DNA, or RNA. The gold core is considered to be non-toxic and the therapeutic payload can be forced to be released from the conjugate due to their photo-physical properties. Drugs can easily be attached through ionic or covalent bonds, or through adhesion. Like for many nanodrugs, PEG can be attached to the surface of metallic nanoparticles to increase stability and circulation time, in addition to other targeting agents [11]. Sodium citrate can also be used as a reducing agent for gold formation, and a stabilizer to avoid aggregation during synthesis [13]. Currently, one phase I clinical trial using tumor necrosis factor α (TNFα) bound to colloidal gold is ongoing to treat advanced solid tumors such as sarcomas and melanomas [14]. Superparamagnetic iron oxide (Fe_3O_4) nanoparticles are also under development and require the use of local hyperthermia or oscillation strategies to deliver conjugated drugs. Magnetic fields can also be used to guide the drug to the intended target area within the body. Unfortunately, their potential clinical use is not presently understood due to the acute *in vivo* toxicity [15]. Moreover, this class of particles is being thoroughly investigated for their use in imaging and theranostics (diagnostics and therapy), but this is beyond the scope of the review.

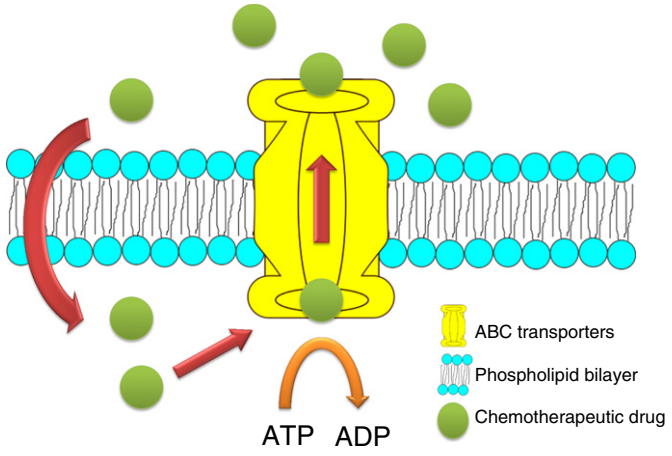

Fig. 3. Upregulation of ABC transporters on cancer cell membranes effectively removes chemotherapeutic drugs and cytotoxic agents as a means of drug resistance.

3. Mechanisms of drug resistance

3.1. Multidrug resistance mechanisms

Multidrug resistance (MDR) is the term used to describe the resistance of cancer to related and unrelated classes of chemotherapeutic drugs and is currently one of the biggest challenges to overcome. Initially, patients may have either a partial or complete response to the first line of treatment but eventually exhibit cancer progression or recurrence. With repeated treatment, tumors often become resistant not only to the specific chemotherapeutic agent being employed, but cross-resistant to both similar and structurally unrelated classes of cytotoxic drugs [16–18].

3.1.1. Efflux pump-mediated MDR

The increased activity of drug efflux occurs primarily through the ATP-binding cassette (ABC) superfamily. ABC transport molecules are typically expressed on the plasma membrane and on the membrane of cellular vesicles and are used to extrude toxins and other foreign substances from the cell. The ABC transporters are transmembrane proteins that use the energy from ATP hydrolysis to shuttle substrates across the membrane. Thirteen of the 48 known ABC transporters contribute to MDR [16]. The first discovery was the *mdr 1* gene that encodes the high molecular weight P-glycoprotein (P-gp/ABCB1), which is amplified in drug-resistant cells and leads to a decrease in drug accumulation [19]. Other proteins including multidrug resistance proteins (MRPs/ABCC) and breast cancer resistance protein (BCRP/ABCG2) are also upregulated in cancer cells and effectively remove cytotoxic agents including Doxorubicin [20–23] and Paclitaxel [24–27] greatly decreasing their concentration within tumor cells, as shown in Fig. 3 [28].

3.1.2. Efflux pump-independent MDR

Additional mechanisms of MDR include decreased drug influx, activation of DNA repair, metabolic modification and detoxification, and altered expression of apoptosis-associated proteins and tumor suppressors, namely mutations in p53 [16]. Normal cells have several repair mechanisms including base excision repair for single strand breaks, homologous recombination and non-homologous end joining repair for double strand breaks, and nucleotide excision repair for mismatches, insertions, and deletions. These mechanisms are used to prevent the transmission of damaged DNA and to avoid malignant transformation [29]. If any of these mechanisms fail, apoptosis is activated to eliminate the damaged cells. However, DNA damage response mutants predispose cells to becoming cancerous and affect response to chemotherapy. Cell cycle arrest does not occur when mutations, chromosomal rearrangements, and epigenetic changes are present, even when induced by anticancer therapies that induce DNA damage to cause cytotoxicity [30]. Further, the anti-apoptotic, pro-survival regulator Bcl-2 [31] and nuclear factor kappa B (NF-κB), a transcription factor that controls genes that suppress apoptotic responses [32], are frequently overexpressed in cancer cells and lead to increased survival.

3.2. Tumor cell heterogeneity, clonal selection and expansion as a potential source of drug resistance

It is accepted at the current stage of cancer biology that tumors at the same clinical grade and histological status are genetically heterogeneous and contain subclonal populations [33]. A large-scale whole-exome sequencing study of 160 chronic lymphocytic leukemia (CLL) patients revealed 20 mutated genes and 5 cytogenetic alterations as driver mutations that spanned 7 core signaling pathways. Clonal mutations (drivers) were found in the majority of tumor cells and represent an early event, whereas subclonal mutations were only found in a small number of leukemic cells, representing a later transformation. In patients

who had undergone chemotherapy, a significantly higher number of subclonal (but not clonal) mutations were found indicating that subclonal mutations are increased with treatment. This may be due to the removal of dominant clones by cytotoxic treatment and a subsequent expansion of subclones, as shown in Fig. 4A. Two time points of whole-exome sequencing for 18 of these patients was also performed. In 10 of the 12 patients that underwent treatment between time points, clonal expansion was evident. Conversely, 5 of the 6 untreated individuals remained at equilibrium between populations over several years. For the treated patients that did exhibit subclonal evolution, the somatic driver mutations that expanded were detectable at the first time point and thus could potentially be anticipated in association with treatment [34]. The emergence of resistant subclones following treatment may allow for tumor expansion and recurrence. The ability to target multiple clonal and subclonal mutations simultaneously is a promising strategy for nanomedicine due to the number of attachment sites present on certain classes of nanoplatforms, which are used in nanodrug development.

3.3. Cancer stem cells (CSCs) and drug resistance

Cancer stem cells, also known as tumor initiating cells, are cells that have the capacity to self-renew and to give rise to the heterogeneous lineages that are found within a tumor [35]. Evidence for cancer stem cells dates back to 1971 when it was shown that only 1 in 100 to 1 in 10,000 mouse myeloma cells were able to form colonies [36]. This was confirmed 6 years later in humans when only 1 in 1000 to 1 in 5000 lung cancer, ovarian cancer, or neuroblastoma cells formed colonies in soft agar [37]. However, a fundamental question on whether all cancer cells had a low probability to behave as stem cells or if only a small subset had the ability to proliferate rapidly and form tumors remained [38]. One essential study to answer this question was performed in a group of acute myeloid leukemia (AML) patients in 1997. Dick et al. showed that only a very small subset of cells that were $CD34^{+}CD38^{-}$ had the ability to cause AML in NOD/SCID mice, indicating that subpopulations of cells had differential abilities to proliferate and transfer disease [39]. Tumor initiating cells were later isolated in breast carcinomas, and it was shown that only a subset of cells could be serially passaged and gave rise to both phenotypically identical and diverse cells consistent with those found in the initial tumor [40]. Cancer stem cells have also been isolated from medulloblastoma, glioblastoma [41], ependymoma [42], colon cancer [43,44], chronic and acute myeloid leukemia [45], pancreatic cancer [46], and head and neck squamous cell carcinomas [47]. However, the origins of these CSCs, i.e. from normal stem cells versus progenitor cells, is still not clear and may vary from tumor to tumor or by tumor type [48]. One key feature of CSCs is the role that they play in resistance to therapy and recurrence. Because chemotherapeutic drugs typically affect frequently dividing cells, CSCs, which are primarily quiescent and have active DNA repair mechanisms, are not harmed. They also express high levels of specific ABC drug transporters, namely ABCB1, ABCG2, and ABCC1, which are known MDR genes in tumor cells, allowing for increased survival. Whereas chemotherapy may be effective against committed tumor cells, the resistant CSCs may survive and repopulate the tumor with self-renewing cells and variably differentiated offspring, as shown in Fig. 4B [49]. The ability to eradicate these CSCs with specific drugs is crucial to prevent tumor repopulation and recurrence.

3.4. Activation of alternate receptors and pathways in cancer as a response to treatment

Therapeutic approaches based on molecular pathways are currently targeting commonly upregulated pathways in order to prevent compensatory pathways. Small molecular inhibitors and monoclonal antibodies show promise in clinical trials and during initial cancer treatment, but resistance is inevitable. A number of intrinsic and acquired resistance mechanisms have been well studied. The activation of alternate receptors, such as c-met and insulin-like growth factor 1 receptor (IGF-1R), in response to anti-epidermal growth factor receptor (EGFR) therapies, is a common adaptation that cancer cells exhibit. The use of other tyrosine kinase receptors allows the cells to bypass the effects of the drug and continue anti-apoptotic, proliferative downstream signaling, in this case, the activation of the phospho-inositide 3-kinase (PI3K) pathway. Antiangiogenic strategies based on VEGFR modulation have also shown resistance through multiple mechanisms. An initial response to anti-VEGF therapy often results in a hypoxia-triggered upregulation of non-VEGF angiogenic factors, such as those in the fibroblast growth factor (FGF) family, and a recurrence of angiogenesis [50]. Thus, drugs that only target one pathway can lead to the reinforcement of alternate pathways that are beneficial to the tumor and may contribute to MDR. Designing nanomedicines that can target multiple pathways at once is ideal for reducing this form of MDR.

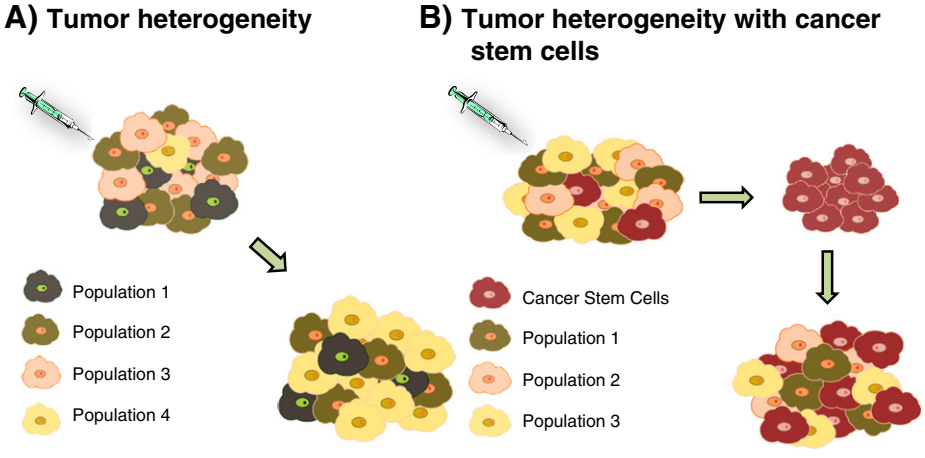

Fig. 4. Two alternate drug resistance mechanisms. A. A heterogeneous population of cancer cells is shown in the top left. Following administration of treatment, Population 3 is completely eliminated while a subpopulation, Population 4, emerges as a dominant clone (bottom right). B. A heterogeneous population of cancer cells, including cancer stem cells, is shown in the top left. After administration of treatment, only the resistant cancer stem cells are seen (top right). After time, the cancer stem cells are able to repopulate the tumor with all previous cell populations present before treatment (bottom right).

3.5. Intrinsic and acquired mutations

Intrinsic mutations have been shown to have a large impact on response to therapy. Somatic mutations resulting in a gain-of-function activation of the tyrosine kinase domain of EGFR in a cohort of non-small cell lung cancer (NSCLC) were discovered in 35% of the enrolled patients and were shown to significantly impact response rates to gefitinib (55%) [51]. In-frame deletions in exon 19 and single missense mutations account for approximately 90% of the EGFR mutations, and result in an increased sensitivity to both small molecule inhibitors, gefitinib and erlotinib [52–54]. Nearly 70% of patients with a mutation (found in 10–25% of all NSCLC patients) respond to therapy compared to only a 10% response rate when no mutations are present [55,56]. However, acquired resistance to therapy is associated with a secondary mutation in exon 20 that leads to substitution of methionine for threonine at position 790M (T790M) in the kinase domain, preventing the binding of erlotinib [57]. Subsequent studies of different patients with acquired T790M mutations revealed that this mutation was not present in untreated tumor samples. Further, the resistance was not found to be associated with KRAS mutations that can cause primary resistance and transfection of cells *in vitro* conferred resistance to gefitinib and erlotinib in normally sensitive cells [58]. An acquired mutation, S492R, in the EGFR ectodomain has also been identified in colorectal cancer patients following treatment with cetuximab that prevents the binding of the antibody. Interestingly, it does not affect the binding ability of a different anti-EGFR mAb, panitumumab [59]. Together, these mutations, whether intrinsic or acquired, allow these cancer cells to avoid the effects of cytotoxic agents and to survive. Thus, nanodrugs designed to knockdown both wild-type and mutated genes are necessary to overcome these compensatory mechanisms. Antisense oligonucleotides attached to polymalic acid biopolymer that block wild-type EGFR and mutated EGFRvIII receptor synthesis have been successfully used to treat triple negative breast cancer [60] employing a strategy based on acquired mutations by the MDA-MB-468 breast cancer cell line.

3.6. Tumor microenvironment and its contribution to MDR

Solid tumors are found within a microenvironment that is comprised of cancer cells and stromal cells (including fibroblasts and immune cells), embedded in an extracellular matrix. This stroma can affect malignant transformation, plays a role in tumor cell invasion and metastasis, and has an impact on drug sensitivity. The tumor stroma has an increased number of fibroblasts (and also myofibroblasts) that synthesize growth factors, chemokines, and adhesion molecules. A representative figure of the complexity of this microenvironment is shown in Fig. 5. The interactions between the cancer cells and these factors can affect the sensitivity of the cells to apoptosis and their response to chemotherapy, and is known as cell adhesion-mediated drug resistance (CAM-DR) [61]. Adhesion of myeloma cells to fibronectin through β1 integrins, whose activation is known to influence apoptosis and cell growth, results in CAM-DR. The adhesion leads to a G1 arrest associated with increased p27kip1 expression and inhibition of cyclin A and E kinase activity; disruption of this interaction returns the tumor cells to a drug-sensitive state [62]. Tumor cells also form polarized, three-dimensional structures through interactions with the basement membrane and ligation of β4 integrins, which regulate polarity and NF-κB activation. These cells become resistant to apoptosis-inducing agents, likely due to the effects on NF-κB [63]. The pH of the tumor microenvironment can also influence the effectiveness of cytotoxic drugs and may inhibit the active transport of some therapeutics [61,64]. The extracellular pH in tumors is acidic and the intracellular pH is neutral to basic. Thus, weakly basic drugs, such as Doxorubicin, are protonated and have reduced cellular uptake [65]. Weakly acidic drugs, such as cyclophosphamide, tend to concentrate in neutral extracellular space [66]. Drug distribution is also affected by the composition and organization of the extracellular matrix [67]. Tumors with a well-organized collagen network prevent some high-molecular weight drugs from penetrating when compared to a poorly organized collagen structure [68]. Further, the tumor microenvironment can create hypoxic situations in which tumor tissue has a diminished oxygen supply that can contribute to MDR [69]. These areas result from abnormal angiogenesis or from the compression/closing of blood vessels by cancer cells [70]. This reduced blood flow may lower concentrations of chemotherapeutics in hypoxic cells [71]. In addition, hypoxia can lead to the activation of genes associated with angiogenesis, survival, and glycolysis through the transcription factor hypoxia-inducible factor 1 (HIF-1) and may contribute to a drug-resistant phenotype [61,72]. HIF-1 transcriptional activity also enhances metabolism, proliferation, invasion, and metastasis by the tumor cells. These hypoxic cells often revert to aerobic metabolism for the production of ATP (the Warburg effect) [73,74] to maintain enough energy to continue to thrive. Interleukin IL-17 is the major effector cytokine of TH17 cells, a subtype of adaptive immunity cells. Tumors resistant to treatment with antibodies to VEGF were rendered sensitive in IL-17 receptor (IL-17R)-knockout hosts deficient in TH17 effector function. Furthermore, pharmacological blockade of TH17 cell function sensitized resistant tumors to therapy with antibodies to VEGF. These findings indicate that IL-17 promotes tumor resistance to VEGF inhibition, suggesting that immunomodulatory strategies could improve the efficacy of anti-angiogenic therapy [75].

Overall, the tumor microenvironment can often provide a physical and chemical network to allow the cancer cells to survive, proliferate, and avoid cytotoxic agents. Thus, the blockage of cancer specific extracellular matrix protein synthesis may lead to physiological normalization of tumor tissue structure (vascular supply) and potential reduction of resistance to conventional chemotherapy [76,77]. Modulation of various aspects of the tumor microenvironment may prove to be an effective strategy in the destruction of the tumor support system that allows cancer cells to survive.

4. Evaluation of nano-drug delivery mechanisms and their potential moieties to treat MDR cancers

4.1. Passive transport and enhanced permeability and retention (EPR) effect

A number of different nanoparticles rely on the characteristics of the tumor for drug accumulation and thus are considered to be passively targeted. During tumor formation, rapid and imperfect angiogenesis occurs, creating leaky blood vessels. Further, these tumors have dysfunctional lymphatic drainage, which also results in drug accumulation [78]. Nanoparticles take advantage of this EPR effect, which allows these drug carriers to accumulate inside of the tumor. However, it has been shown that there are inconsistencies in vascular pore size both within a tumor and between different tumor types. This can lead to an unpredictable accumulation of the drug in only certain areas of the tumor, or possibly not at all. The EPR effect is also influenced by the surrounding stroma, the location and size of the tumor, the amount of infiltration by macrophages (which can internalize liposomes resulting in macrophage toxicity), patient characteristics such as age and gender, and additional medications. Currently, the available clinical data relate to passively targeted liposomes, but a number of actively targeting nanoparticles are also in clinical development [79]. Due to the unpredictability of the EPR effect and the ineffectiveness against non-solid tumors such as leukemia, a greater focus on targeted therapy may be necessary in the future. Despite the fact that the EPR-based targeting is only passive and may be unpredictable, nanodrugs consistently have a better accumulation within a tumor than free drugs including Paclitaxel [80], rapamycin [81], thiostrepton [8], Doxorubicin [82], and Salinomycin [83], among countless others.

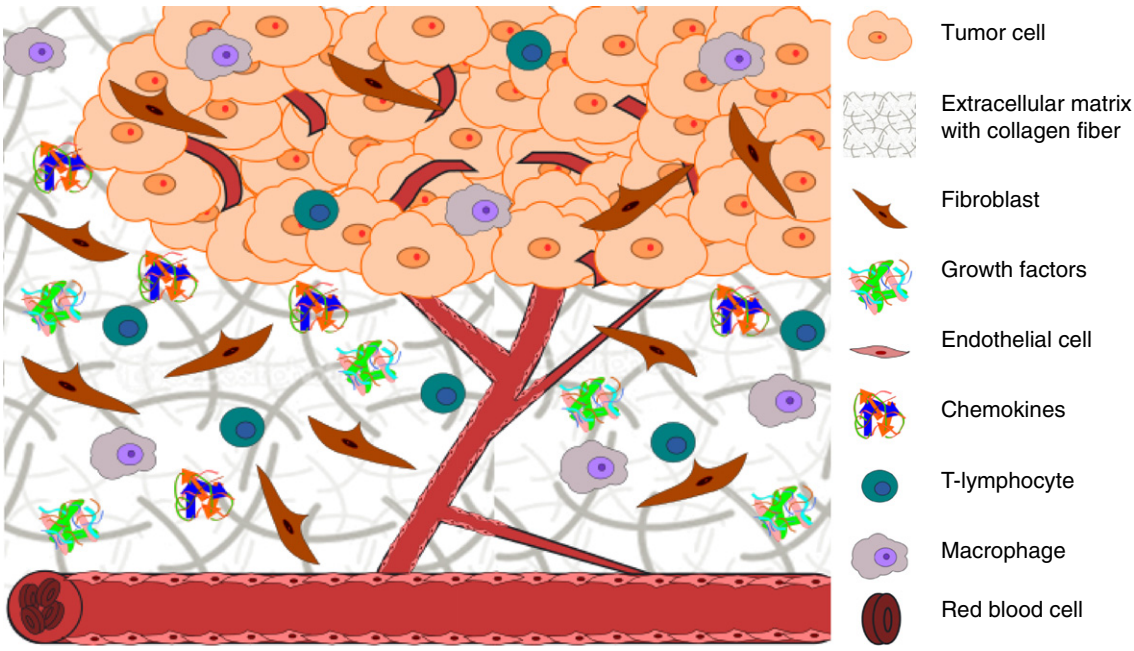

Fig. 5. Representative example of the complexity of the tumor microenvironment and its interactions with tumor cells.

4.2. The addition of polyethylene glycol (PEG) to increase blood circulation time

The conjugation of polyethylene glycol (PEG) to nanoparticles has been shown to increase circulation time *in vivo*. A number of potential mechanisms have been proposed to explain this observation although it is still not completely understood. These mechanisms include: reduced opsonization, promotion of the adsorption of proteins which may mask the particle (dysopsonization), aggregation prevention, steric hindrance to block the binding of reticuloendothelial system (RES) cells, which are responsible for the clearance of nanoparticles, and stabilization of lipid layers. Recently, Gottstein et al. used a mathematical model combined with high throughput flow cytometry and quantitative confocal microscopy to confirm a general trend of reduced internalization by RES cells [84]. Since chemotherapeutic agents are typically low in molecular weight, they are rapidly cleared from the body and often suffer from a short half-life in blood. The need to improve drug accumulation in the tumor site during treatment, especially for resistant tumors, relies heavily on the stability of the drug in plasma, which is greatly increased with nanomedicines. The first PEGylated nanoparticle approved in the United States and Europe is liposomal Doxorubicin (Doxil®/Caelyx® [PLD]) for the treatment of Kaposi's sarcoma [85], recurrent ovarian cancer [86] and multiple myeloma [87], with additional clinical trials ongoing for metastatic breast [88], and hormone refractory prostate cancer [89]. The conjugation of PEG to liposomes [1,90], polymer-based nanoparticles and micelles [7,91], dendrimers [92], gold nanoparticles [11,13], and superparamagnetic iron oxide [93] is now a common practice to improve circulation time and avoid clearance. However, it is important to note that there are still possible negative side effects including an immunological response through complement activation, toxicity of side products, and possible accumulation of PEG due to its non-biodegradability [94].

4.3. Active targeting agents to increase drug accumulation and overcome MDR

4.3.1. Antibodies and their fragments specifically target cancer cells

A number of mAbs have been approved for the treatment of various cancers including rituximab (Rituxan) for non-Hodgkin's lymphoma [95], the anti-HER2 trastuzumab (Herceptin) [96], the anti-VEGF bevacizumab (Avastin) to inhibit angiogenesis [97], and the anti-EGFR cetuximab [98], along with many others that are either already approved or are undergoing clinical trials. These therapeutic antibodies, as well as targeting antibodies such as 2C5 [2] and anti-transferrin receptor mAbs [99–101], can be conjugated to various nanoparticles to improve efficacy and increase binding affinity to the cancer cells. The increased specificity results in a higher accumulation of drug within the tumor rather than other vital organs, reducing toxicity and making the drug better equipped to overcome MDR. The use of whole antibodies is considered advantageous due to the presence of two binding sites and increased stability during long-term storage. However, the intact Fc domain may also bind to the Fc receptors on normal cells causing an activated signaling cascade that may result in increased immunogenicity [102]. Thus, the specificity of the targeting/therapeutic antibody is crucial to avoid toxicity to normal tissue.

4.3.2. Nucleic acid aptamers (single stranded DNA or RNA oligonucleotides)

Aptamers are nucleic acid ligands that can bind with high affinity and specificity. Strategies have been developed to isolate and enrich cancer cell-specific internalizing aptamers, and they have been successfully conjugated to a number of different nanoparticles. They are considered to have high affinity and low immunogenicity but some drawbacks include lack of flexibility, length (typically 75–100 nucleotides), and *in vivo* nuclease stability. Currently, all nanoparticles using aptamers as target agents are still in the preclinical phase [103].

4.3.3. Receptor ligands (peptides) as non-immunogenic targeting agents

The conjugation of peptides as targeting agents is favorable due to their small size, the ease of synthesis, and their typical non-immunogenicity. Tumor homing peptides include those with an RGD sequence motif, i.e. a binding motif for integrins, such as $\alpha v\beta 3$, which is specifically expressed on tumor endothelia [104], and those that have a form of aminopeptidase N (CD13) that binds peptides with the NGR motif. Delivery of TNFα using both RGD and NGR peptides has shown to decrease the effective dose by up to 1000-fold [105] and an NGR-hTNF peptide is currently in phase III clinical trials [106]. Further, these peptides may not only play a role in homing but in tumor-

penetration, as is the case with iRGD, because they allow for the exit from blood vessels and can activate a tissue-specific transport pathway [105], which is a big advantage in treating resistant tumors. Despite their potential, it is important to note that peptides such as RGD can bind to other integrins on normal tissue making specificity of the peptide a crucial consideration [102]. Moreover, most peptides on nanoconjugates rely on polyvalency to achieve optimal cell binding. Polyvalency depends on geometry and density of targeted receptors that are, however, difficult to mirror on nanoparticles [87].

4.4. Enhanced endosomal escape to improve efficacy of the drug once internalized

Nanoparticles have been shown to be internalized through clathrin-dependent and clathrin-independent endocytosis depending on the cell type and the composition of the cell surface. The addition of antibodies and targeting ligands is aimed at increasing receptor-mediated endocytosis. However, once inside the cell, these nanoparticles may either fuse with lysosomes or be recycled back to the cell surface, making endosome escape a key limitation [107]. To overcome this barrier, Pittella et al. synthesized a nanocarrier system composed of calcium phosphate and comprising PEG and charge-conversion polymer (CCP) to deliver siRNA. The PEG-CCP is an endosomal escape unit that induces endosomal membrane destabilization by producing polycation through degradation of the flanking *cis*-aconitylamide of CCD in the acidic endosome environment. Rapid endosomal escape was confirmed using confocal laser scanning microscopy, and ~80% VEGF mRNA knockdown in pancreatic cancer cells was achieved [108]. Ding et al. also took advantage of the acidic endosome environment and conjugated H_2N-Leu-Leu-Leu-OH (LLL) to our polymalic acid based nanopolymer (P/LLL). At physiological pH 7.4, the P/LLL conjugates were inactive but at pH 5–5.5 (the range of acidification in late endosomes and lysosomes), activity was upregulated and membrane disruption allowed for endosomal escape of the nanoconjugate [76]. Biological activity of nanoparticles often relies heavily on the ability to escape the endosome and enter the cytosol, making endosome escape units an area not to be overlooked.

5. Specific resistance mechanisms overcome by nanomedicine

5.1. Evasion and down-regulation of drug efflux pumps to treat MDR tumors

It is agreed that chemotherapeutics bound as nanoconjugates or encapsulated into nanoparticles evade the capture of ABC drug efflux pumps. This is so because the chemically bound or encapsulated drugs are not physically recognized as substrates by the ABC efflux systems. After crossing the efflux containing membranes on endosomal delivery pathway, the free chemotherapeutics are released into the perinuclear region of the cytoplasm and can unfold their activities. Alternate methods of entry into the target cell uses virus-derived TAT peptides, non-specific cell penetrating peptides that interact strongly with phosphate head groups of phospholipids at both sides of the lipid bilayer, followed by insertion of charged side chains that form a transient pore, which allows the translocation of the TAT peptides. Small molecules attached to peptides can be internalized through this mechanism, whereas TAT-polymer conjugate can be taken up through energy-dependent endocytosis or macropinocytosis [18]. A novel polymeric micelle consisting of Doxorubicin and two block copolymers, one conjugated to TAT, has been produced. The micelle surface hides the TAT during circulation and only exposes it at a slightly acidic tumor extracellular pH to allow for TAT-induced internalization into cancerous cells. The micelle core then disintegrates in the early endosomal pH of the cells to release Doxorubicin. Further, the ionization of the block copolymers aids in disrupting the endosomal membrane, allowing the drug to accumulate in the cytosol. Regression of tumors was apparent in xenograft models of human ovarian tumor drug-resistant A2780/AD, human breast tumor MCF-7, human lung tumor A549, and human epidermoid tumor KB using this drug as a non-specific targeting agent [109]. Human gliomas are also known to have enhanced activity of drug efflux pumps. The multidrug-resistance-associated protein (MRP), an ABC membrane transporter not dependent on P-glycoprotein, is highly expressed in the severely drug resistant glioma cell line T98G (4.5 fold higher than drug-sensitive U87MG) [110]. Currently, the most effective strategy to treat glial cells is temozolomide (TMZ), a pro-drug releasing a DNA alkylating agent, combined with radiation. However, TMZ is toxic, has severe side effects, and frequently encounters tumor drug resistance. Free TMZ, which has a short half-life of 1.8 h, proved to be ineffective *in vitro* against the resistant T98G cell line, as well as two human breast cancer lines MDA-MB-231 and MDA-MB-468, although U87MG was sensitive. A multifunctional targetable nanoconjugate of TMZ hydrazide was synthesized using a poly (β-L-malic acid) platform conjugated with the targeting mAb to human transferrin receptor (TfR) for receptor-mediated endocytosis, LLL for pH-dependent endosomal membrane disruption, and PEG for protection. The conjugated TMZ had an increased half-life of 5–7 h. Delivery of PMLA-TMZ conjugate to T98G, MDA-MB-231, and MDA-MB-468 was able to effectively overcome the resistance of free TMZ and significantly reduced cell viability as shown in Fig. 6. The greater drug accumulation was due to the avoidance of the drug efflux pump mechanism via receptor-mediated endocytosis [111]. In addition to T98G, the human glioblastoma cell line U251 is known to have MDR due to the high expression of P-glycoprotein and other ABC transporters. Another version of PMLA containing PEG, with Doxorubicin conjugated via pH-sensitive hydrazone linkage, was able to effectively inhibit growth of U251 more than Doxorubicin alone due to the modified drug uptake mechanism that evaded drug efflux pumps [112].

Direct targeting of the upregulated P-glycoprotein while simultaneously treating with anti-cancer therapeutics is yet another strategy that nanomedicine can employ. The use of P-glycoproteins inhibitors is an attractive field but has unfortunately resulted in numerous failures in the clinic to date. Inhibitors including verapamil, a calcium channel blocker, and cyclosporine, an immunosuppressant, are actually substrates for P-glycoprotein and compete for efflux with the chemotherapeutic drugs. Their lack of specificity requires a large dose to achieve clinical inhibition and thus results in toxicity. Numerous modifications of these inhibitors have appeared as promising candidates, but clinical trials continue to fail [113]. However, the use of these molecules in relation to nanomedicines is still an attractive strategy due to increased targeting and accumulation. For example, Wu et al. used a liposome co-encapsulating Doxorubicin and verapamil and conjugated to transferrin, to effectively overcome MDR in K562 leukemia cells [114]. Another group encapsulated curcumin, known as P-glycoprotein pump inhibitor, and Paclitaxel in a nanoemulsion of flaxseed oil to effectively treat wild-type and drug resistant SKOV3 ovarian tumor cells [115]. Further, liposomal anti-MRP-1 and anti-Bcl2 siRNA in combination with Doxorubicin were used to suppress pump and non-pump mediated cellular resistance and to cause death in MDR lung cancer cells [116]. Finally, stealth liposomes containing PSC 833 (Valspodar, a cyclosporin A analog that is more effective) and Doxorubicin were able to reverse MDR in the Doxorubicin-resistant human breast cancer cell line T47D/TAMR-6 [117].

5.2. Targeting cancer stem cells to overcome MDR and prevent recurrence

Cancer stem cells are more resistant to treatment and conventional chemotherapeutics often fail to destroy them. CSC resistance is achieved through increased Wnt/β-catenin and Notch signaling, high levels of ATP cassette reporters, altered DNA repair mechanisms, and slow proliferation rate [118]. A study of osteosarcoma cell lines showed that an anti-cancer drug Salinomycin could suppress tumor cells *in vitro*

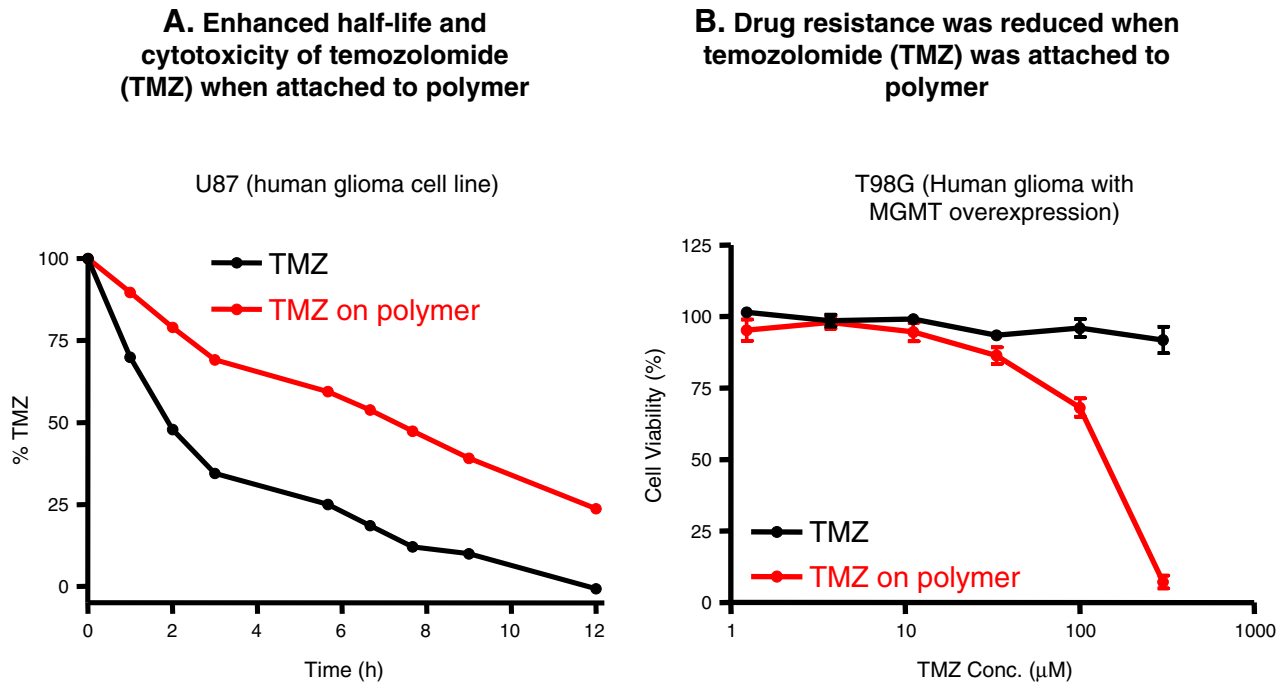

Fig. 6. The conjugation of TMZ on polymalic acid nanobiopolymer increases half-life and overcomes resistance in human glioma cell line T98G. A. Half-life of TMZ was increased from 1.8 to 7.4 h (>4 times) after attachment to polymer. B. Attachment of TMZ to polymer resulted in overcoming the inherent resistance of T98G cells compared to TMZ alone. Reproduced from Ref. [111].

and *in vivo* by targeting CSC, potentially through the Wnt/β-catenin signaling pathway [119]. Zhang et al. loaded Salinomycin on PEGylated polymeric micelles to effectively target CSC (CD44+/CD24−) isolated from breast cancer cell line MCF-7, and these micelles were more effective than Salinomycin alone *in vivo*. A combination treatment with octreotide-modified-Paclitaxel-PEG polymeric micelles was shown to enhance the binding to somatostatin receptors, which are enhanced in many cancers, to eradicate both tumor cells and CSC *in vivo* via receptor-mediated endocytosis [83]. The knockdown of other CSC related pathways including tissue transglutaminase (TG2) by gene silencing using liposomal anti-TG2 siRNA combined with gemcitabine to treat Panc-28 pancreatic cancer cells was efficacious in reducing tumor growth and preventing metastasis [120]. Additional stem cell markers including CD44 [38,121] and CD133 [121] have been associated with drug resistance and may serve as potential targets for future nanodrugs to eliminate CSCs and prevent recurrence.

5.3. Preventing the cross talk of cancer cells and their microenvironment

Prevention of the cross talk between cancer cells and supporting stroma and vasculature, which promotes cell growth and prevents apoptosis, is an attractive strategy for overcoming resistance to therapy. Mature B-cell malignancies are known to interact via CXCR4 signaling, a G-coupled protein receptor, found on hematopoietic and epithelial cancer cells. Stromal cells located in the bone marrow microenvironment secrete stromal cell-derived factor 1 (SDF-1/CXCL12), the ligand for CXCR4. This signaling recruits the cancer cells to the bone marrow where they receive additional growth and drug resistance signals. Antagonists of CXCR4 can disrupt these interactions, forcing the leukemia cells back into circulation where they are more susceptible to chemotherapeutic drugs [122], and may serve as crucial additions on nanodrugs. Attempts to actually target and destroy tumor stromal cells have also been performed to prevent their contribution to tumor growth. Tumor-related stromal cells express high levels of platelet-derived growth factor receptor-β (PDGFR-β). A unique nanocarrier was made using albumin and a PDGFR-β recognizing cyclin peptide conjugated to Doxorubicin through an acid-sensitive hydrazone linkage. *In vivo*, the drug rapidly accumulated in PDGFR-β expressing cells in C26 murine colon cancer and significantly reduced tumor growth; free Doxorubicin was not as effective and resulted in loss of body weight [123]. Interfering with the signaling of the microenvironment or even potentially eliminating key stromal contributors of anti-apoptotic, pro-growth, and pro-angiogenesis signals using a targeted nanotherapy are promising approaches.

5.4. Modifying the immune response to improve treatment of MDR cancers

Immune response modification can occur either through inhibition or enhancement. Several groups have used siRNA, frequently in cationic liposomes, to downregulate essential immune transcription factors, proinflammatory cytokine production, especially TNF-α, or cellular receptors to prevent cell activation. Both siRNA and various nanoparticles can elicit an interferon-mediated immune response. Therefore, it is not only the inhibitory effect of the siRNA, but also the immunostimulatory effect of the treatment that leads to a reduction in tumor size [124]. Using siRNAs to elicit an antitumor effect have shown to be effective. Pertinent examples include using Bcl2-specific siRNA with 5′-triphosphate ends against melanoma to silence Bcl2 and enhance activity of natural killer cells and interferon through innate cell activation via Rig-1 [125]; using a toll-like receptor (TLR)9 agonist, Stat3, siRNA synthetically linked to a CpG oligonucleotide to inhibit expression in dendritic cells, macrophages and B cells, leading to the activation of tumor-associated immune cells and an anti-tumor immune response to mouse melanoma and colon cancer [126]; and using a bifunctional siRNA complexed with PEGylated liposomes to inhibit HPV16 E6/E7 mRNA and to activate immune response cells via TLR7 to effectively inhibit TC-1 tumors *in vivo* [127]. In addition to the use of siRNAs, other nanomedicine approaches for altering immune response have been explored. An antibody cytokine fusion protein consisting of the immunostimulatory cytokine interleukin-2 (IL-2)

genetically fused to an antibody specific for human HER2/neu was covalently attached to a polymalic acid (PMLA) backbone. The drug also contained antisense oligonucleotides against α4 and β1 chains of vascular tumor protein laminin-411 to block angiogenesis. Treatment of immunocompetent mice bearing murine mammary tumors expressing human HER2/neu resulted in significant increases of IgG1 and IgG2a, indicative of a humoral (T_H2) and cell-mediated (T_H1) immune response, as well as decreased tumor growth and longer survival [128]. With the increase of resistance to a number of therapeutics, the use of immunostimulatory drugs may prove to be advantageous in avoiding and overcoming drug resistance in the future.

6. Recent progress in overcoming tumor resistance by using nanomedicines

Engineering of multifunctional nanodrugs demonstrates new possibilities to overcoming drug resistance that were not possible with conventional therapy or combination of different current cancer treatments. Recent experimental progress in overcoming resistance by using nanomedicines *in vitro* and *in vivo* is summarized in Table 1. Nanocarriers can display their own anti-drug resistance activity aside from actions by their cargo. This has been shown for α-tocopheryl PEG1000 succinate [129,130] and poly[bis(2-hydroxylethyl)-disulfide-

Table 1
Recent progress in overcoming tumor drug resistance by using nanomedicines.

Tumor type	Nanomedicines	Active groups	Action mechanism	Ref.
Docetaxel (DTX)-resistant human ovarian A2780/T. *In vitro model*	D-α-Tocopheryl polyethylene glycol 1000-block-poly(β-amino ester) containing micellar nanoparticle	D-α-Tocopheryl -polyethylene glycol, docetaxel	Inhibition of P-gp to decrease DTX efflux; DTX Inhibition of cell division	[133]
H460/TaxR human non-small cell lung cancer overexpressing P-gp *In vitro model*	D-α-Tocopheryl polyethylene glycol 1000 succinate containing micellar nanoparticle	D-α-Tocopheryl polyethylene glycol 1000 succinate; paclitaxel (PTX), fluorouracil (5-FU)	Inhibition of P-glycoprotein by Tocopheryl polyethylene glycol 1000 succinate; Inhibition of cell division by PTX; irreversible inhibition of thymidylate synthase; synergism of PTX/5-FU.	[140]
Human MCF7/ADR tumor on BALB/c nude mice. *In vivo* breast cancer model	Poly[bis(2-hydroxylethyl)-disulfide-diacrylate-β-tetraethylenepentamine]–polycaprolactone copolymer (PBD–PCL) containing micelle nanoparticles	shRNA to Survivin, PBD–PCL, Doxorubicin	Inhibition of: P-glycoprotein; inhibition of glutathione S-transferase, intercalation into DNA	[137]
$CD138^-$ $CD34^-$ cells isolated from a human U266 multiple myeloma cell line inoculated in mice with non-obese diabetic/severe combined immunodeficiency (NOD/SCID). *In vivo* model	Polyoxypropylene chain and oleic acid coated iron oxide NPs	Anti-ABCG2 antibody, PTX	Antibody blocking of ABCG2 to inhibit PTX resistance; PTX inhibition of cell division	[138,144]
Human lung adenocarcinoma A549-Bcl-2 cells *In vitro model*	Micelleplexes	siRNA to BCl-2, PTX	Downregulation of Bcl-2; PTX Inhibition of cell division	[129]
CAL27 cisplatin-resistant human oral cancer cells (CAR cells), *In vitro model*	PLGA nanoparticles	Curcumin, cisplatin	Pt-DNA crosslinks; MDR-1 suppression; Triggering of Apoptosis	[131]
Human breast cancer MDA-MB-231 cells inoculated into BALB/c nu/nu mice. Xenogeneic *in vivo* model	PLGA nanoparticles conjugated to Anti-CD133	Anti-CD133, PTX	Targeting tumor initiating cells $CD133^+$; PTX inhibition of cell division	[134]
GS5 glioblastoma multiforme cells (obtained from human U87GM cells enriched by stem cells) injected intracranially in rats. *In vivo* model	PGLA nanoparticles Treatment by convection-enhanced delivery (CED)	Dithiazanine iodide (DI)	DI displays toxicity towards brain cancer stem cells	[135,145]
Rat F98 glioblastoma inoculated on Fischer 344 rats. Orthotopic syngeneic *in vivo* model	PGLA-chitosan	Carmustine (BCNU), O(6)-benzylguanine (BG)	BCNU for DNA alkylating and crosslinking; BG for inhibition of O(6)-methylguanine-DNA-methyl transferase (MGMT)	[130]
Chemotherapy- and antiandrogen-resistant mAR+/GPRC6A+ DU-145 human prostate carcinoma cells. *In vivo* model	Gold nanoparticles	Multiple α-Bic- and β-Bic antiandrogens	Multivalent binding to androgen receptor and to G-protein coupled receptor (GPRC6A); The antiandrogens inhibit binding of androgen	[143,146]
Human breast MDA-MB-231 and MDA-MB-468 cell lines, and brain cancer cell lines U87MG and T98G *In vitro model*	Polymer–drug conjugate based on poly (β-L-malic-acid) platform	Temozolomide (TMZ)	TMX is a DNA alkylating agent preventing cell division	[110]
Human Lewis lung carcinoma A549 cells subcutaneously inoculated into C57BL/6N mice, *In vivo* model	Nanoliposomes in combination with radiation therapy	Cisplatin (CDDP), Radiation therapy	Cisplatin alkylating and crosslinking DNA; Sensation to radiation lesions	[136]
Human SW480 Colorectal cancer. *In vitro model*	Human serum albumin-based anti-Survivin siRNA delivery in combination with radiation therapy	Anti-Survivin siRNA; Radiation therapy	Knocking down of Survivin promotes apoptosis	[141]
Human melanoma cells HMV-II; Radiation resistance under hypoxic conditions. *In vitro model*	Liposomes in combination with radiation therapy	Pimonidazole (Pmz); Radiation therapy	DNA fragmentation and crosslinking sensitizes for radiation damage	[142]
Human U251 glioblastoma intracranially grown in Nu/Nu rats. *In vivo model*	Magnetic ferric oxide NP in combination with radiation therapy	TRIAL, a type-II transmembrane homotrimeric protein (TNF gene superfamily) Gamma irradiation	Radiation sensitizes for TRAIL induced apoptosis; Sensitization of TRIAL by conjugation to the ferric oxide nanoparticle	[139]
HMLER (shE-cadherin) human breast cancer stem cells (BCSCs) inoculated into mice to treat triple-negative breast cancer. *In vivo model*	Multiwalled carbon nanotubes (MWCNTs) in combination with photothermal (laser) treatment.	Nanotubes without active targeting but with specific permeation into BCSCs; Photothermal treatment.	Thermal therapy promotes rapid MWCNT membrane permeabilization resulting in necrosis of BCSCs and differentiated cancer cells.	[132,147]

diacrylate-β-tetraethylenepentamine]-polycaprolactone copolymer [131] for the inhibition of efflux-dependent mechanisms or carbon nanotubes for membrane permeabilization and sensitization to photo thermal therapy [132]. Drug efflux systems [129–131,133–136], resistance due to presence of CSC [132,137–139], DNA damage/repair [136,140–142], apoptosis signaling pathways [139,141,143], resistance against radiation therapy [136,139,141,142], and resistance against thermal treatment [142] are being studied *in vitro* and *in vivo* as targets for nanomedicines in order to develop the best treatment regimens. Diverse functions and multiple effects are typical for the composition and design of multifunctional nanomedicines. Active cargo, such as siRNA, and antibodies specifically inhibit synthesis or function of proteins, which are key players in resistance mechanisms [134,135,137]. Various chemotherapeutic drugs when delivered as a nanoparticle cargo can bypass drug resistance resulting in higher toxicity for tumor cells with lower toxicity to normal tissues. Examples of the drugs used as part of nanoparticles and nanoconjugates include taxanes [129,130,134,135,137], 5-fluorouracil [140], dithiazanine iodide [135], carmustin, cisplatin, pimonidazole [130,136,142], effectors of enzyme activity [130,140] and signaling, such as antiandrogens and TRAIL [139,143]. Nanoparticles and their cargoes have been found to be sensitizers for killing of differentiated cancer cells and CSCs by radiation therapy [136,139,141,142] and laser hyperthermal therapy [132]. It can be hypothesized that cancer therapy using nanodrugs alone or in combination with radiation/hyperthermia can overcome resistance by delivery of one or several nonrelated therapeutic agents, and modern nanomedicines can afford to deliver all these moieties into the cancer cells.

7. Conclusion and future direction

The lifetime probability of developing cancer for men (45%) and women (38%) is startlingly high and accounts for 1 in 4 deaths in the United States [148]. Despite our vast expanse of knowledge regarding the disease, it continues to progress faster than we can keep up with. A number of underlying mechanisms regarding progression, metastasis, and invasion have been elucidated at both cellular and molecular levels, which serve as promising traits to focus on for nanodrug therapy when surgery, radiation, and chemotherapy are insufficient. Despite these advances that target signaling mechanisms and upregulated genes and proteins, drug resistance remains a key feature of cancer cells and is often acquired even after an initial positive response. Nanomedicines that have increased circulation time, precise multiple targeting mechanisms, enhanced drug accumulation at the tumor site, delivered into the cytoplasm and/or nuclei of cancer cells, and have the ability to carry combinations of therapeutic payloads are attractive treatment options in overcoming MDR. Numerous unique nanodrugs have been created and researched extensively, and are already in clinical development. As additional discoveries and optimizations are achieved, the superiority of nanomedicines over current treatment options and free drugs will continue to increase for the efficient eradication of drug-resistant cancers.

References

[1] F. Perche, V.P. Torchilin, Recent trends in multifunctional liposomal nanocarriers for enhanced tumor targeting, J. Drug Deliv. 2013 (2013) 705265.
[2] V.P. Torchilin, Antinuclear antibodies with nucleosome-restricted specificity for targeted delivery of chemotherapeutic agents, Ther. Deliv. 1 (2010) 257–272.
[3] T. Yang, M.K. Choi, F.D. Cui, S.J. Lee, S.J. Chung, C.K. Shim, D.D. Kim, Antitumor effect of paclitaxel-loaded pegylated immunoliposomes against human breast cancer cells, Pharm. Res. 24 (2007) 2402–2411.
[4] T.M. Allen, P.R. Cullis, Liposomal drug delivery systems: from concept to clinical applications, Adv. Drug Deliv. Rev. 65 (2013) 36–48.
[5] D. Needham, G. Anyarambhatla, G. Kong, M.W. Dewhirst, A new temperature-sensitive liposome for use with mild hyperthermia: characterization and testing in a human tumor xenograft model, Cancer Res. 60 (2000) 1197–1201.
[6] C.M. Hu, L. Zhang, Nanoparticle-based combination therapy toward overcoming drug resistance in cancer, Biochem. Pharmacol. 83 (2012) 1104–1111.
[7] G. Mattheolabakis, B. Rigas, P.P. Constantinides, Nanodelivery strategies in cancer chemotherapy: biological rationale and pharmaceutical perspectives, Nanomedicine (Lond.) 7 (2012) 1577–1590.
[8] K. Cho, X. Wang, S. Nie, Z.G. Chen, D.M. Shin, Therapeutic nanoparticles for drug delivery in cancer, Clin. Cancer Res. 14 (2008) 1310–1316.
[9] T. Minko, Soluble polymer conjugates for drug delivery, Drug Discov. Today Technol. 2 (2005) 15–20.
[10] J. Lim, E.E. Simanek, Triazine dendrimers as drug delivery systems: from synthesis to therapy, Adv. Drug Deliv. Rev. 64 (2012) 826–835.
[11] D.M. Webster, P. Sundaram, M.E. Byrne, Injectable nanomaterials for drug delivery: carriers, targeting moieties, and therapeutics, Eur. J. Pharm. Biopharm. 84 (2013) 1–20.
[12] C. Fabbro, H. Ali-Boucetta, T. Da Ros, K. Kostarelos, A. Bianco, M. Prato, Targeting carbon nanotubes against cancer, Chem. Commun. (Camb.) 48 (2012) 3911–3926.
[13] R. Arvizo, R. Bhattacharya, P. Mukherjee, Gold nanoparticles: opportunities and challenges in nanomedicine, Expert Opin. Drug Deliv. 7 (2010) 753–763.
[14] S.K. Libutti, G.F. Paciotti, A.A. Byrnes, H.R. Alexander, W.E. Gannon, M. Walker, G.D. Seidel, N. Yuldasheva, L. Tamarkin, Phase I and pharmacokinetic studies of CYT-6091, a novel PEGylated colloidal gold-rhTNF nanomedicine, Clin. Cancer Res. 16 (2010) 6139–6149.
[15] S. Bhattacharyya, R.A. Kudgus, R. Bhattacharya, P. Mukherjee, Inorganic nanoparticles in cancer therapy, Pharm. Res. 28 (2011) 237–259.
[16] X. Xue, X.J. Liang, Overcoming drug efflux-based multidrug resistance in cancer with nanotechnology, Chin. J. Cancer 31 (2012) 100–109.
[17] X. Dong, R.J. Mumper, Nanomedicinal strategies to treat multidrug-resistant tumors: current progress, Nanomedicine (Lond.) 5 (2010) 597–615.
[18] A. Shapira, Y.D. Livney, H.J. Broxterman, Y.G. Assaraf, Nanomedicine for targeted cancer therapy: towards the overcoming of drug resistance, Drug Resist. Updat. 14 (2011) 150–163.
[19] C.J. Chen, J.E. Chin, K. Ueda, D.P. Clark, I. Pastan, M.M. Gottesman, I.B. Roninson, Internal duplication and homology with bacterial transport proteins in the mdr1 (P-glycoprotein) gene from multidrug-resistant human cells, Cell 47 (1986) 381–389.
[20] J. van Asperen, O. van Tellingen, F. Tijssen, A.H. Schinkel, J.H. Beijnen, Increased accumulation of doxorubicin and doxorubicinol in cardiac tissue of mice lacking mdr1a P-glycoprotein, Br. J. Cancer 79 (1999) 108–113.
[21] N. Shiraki, A. Hamada, T. Ohmura, J. Tokunaga, N. Oyama, M. Nakano, Increase in doxorubicin cytotoxicity by inhibition of P-glycoprotein activity with lomerizine, Biol. Pharm. Bull. 24 (2001) 555–557.
[22] S. Ye, D.P. MacEachran, J.W. Hamilton, G.A. O'Toole, B.A. Stanton, Chemotoxicity of doxorubicin and surface expression of P-glycoprotein (MDR1) is regulated by the Pseudomonas aeruginosa toxin Cif, Am. J. Physiol. Cell Physiol. 295 (2008) C807–C818.
[23] J. Wang, H. Wang, L. Zhao, S. Fan, Z. Yang, F. Gao, L. Chen, G.G. Xiao, J. Molnár, Q. Wang, Down-regulation of P-glycoprotein is associated with resistance to cisplatin and VP-16 in human lung cancer cell lines, Anticancer Res 30 (2010) 3593–3598.
[24] S.H. Jang, M.G. Wientjes, J.L. Au, Kinetics of P-glycoprotein-mediated efflux of paclitaxel, J. Pharmacol. Exp. Ther. 298 (2001) 1236–1242.
[25] E.M. Kemper, A.E. van Zandbergen, C. Cleypool, H.A. Mos, W. Boogerd, J.H. Beijnen, O. van Tellingen, Increased penetration of paclitaxel into the brain by inhibition of P-glycoprotein, Clin. Cancer Res. 9 (2003) 2849–2855.
[26] H. Enokida, T. Gotanda, S. Oku, Y. Imazono, H. Kubo, T. Hanada, S. Suzuki, K. Inomata, T. Kishiye, Y. Tahara, K. Nishiyama, M. Nakagawa, Reversal of P-glycoprotein-mediated paclitaxel resistance by new synthetic isoprenoids in human bladder cancer cell line, Jpn. J. Cancer Res. 93 (2002) 1037–1046.
[27] M.M. Pires, D. Emmert, C.A. Hrycyna, J. Chmielewski, Inhibition of P-glycoprotein-mediated paclitaxel resistance by reversibly linked quinine homodimers, Mol. Pharmacol. 75 (2009) 92–100.
[28] M.M. Gottesman, T. Fojo, S.E. Bates, Multidrug resistance in cancer: role of ATP-dependent transporters, Nat. Rev. Cancer 2 (2002) 48–58.
[29] C.J. Lord, A. Ashworth, The DNA damage response and cancer therapy, Nature 481 (2012) 287–294.
[30] M. Rebucci, C. Michiels, Molecular aspects of cancer cell resistance to chemotherapy, Biochem. Pharmacol. 85 (2013) 1219–1226.
[31] V. Kirkin, S. Joos, M. Zörnig, The role of bcl-2 family members in tumorigenesis, Biochim. Biophys. Acta 1644 (2004) 229–249.
[32] M. Bentires-Alj, V. Barbu, M. Fillet, A. Chariot, B. Relic, N. Jacobs, J. Gielen, M.P. Merville, V. Bours, NF-κB transcription factor induces drug resistance through MDR1 expression in cancer cells, Oncogene 22 (2003) 90–97.
[33] M. Gerlinger, A.J. Rowan, S. Horswell, J. Larkin, D. Endesfelder, E. Gronroos, P. Martinez, N. Matthews, A. Stewart, P. Tarpey, I. Varela, B. Phillimore, S. Begum, N.Q. McDonald, A. Butler, D. Jones, K. Raine, C. Latimer, C.R. Santos, M. Nohadani, A.C. Eklund, B. Spencer-Dene, G. Clark, L. Pickering, G. Stamp, M. Gore, Z. Szallasi, J. Downward, P.A. Futreal, C. Swanton, Intratumor heterogeneity and branched evolution revealed by multiregion sequencing, N. Engl. J. Med. 366 (2012) 883–892.
[34] D.A. Landau, S.L. Carter, P. Stojanov, A. McKenna, K. Stevenson, M.S. Lawrence, C. Sougnez, C. Stewart, A. Sivachenko, L. Wang, Y. Wan, W. Zhang, S.A. Shukla, A. Vartanov, S.M. Fernandes, G. Saksena, K. Cibulskis, B. Tesar, S. Gabriel, N. Hacohen, M. Meyerson, E.S. Lander, D. Neuberg, J.R. Brown, G. Getz, C.J. Wu, Evolution and impact of subclonal mutations in chronic lymphocytic leukemia, Cell 152 (2013) 714–726.
[35] M.F. Clarke, J.E. Dick, P.B. Dirks, C.J. Eaves, C.H. Jamieson, D.L. Jones, J. Visvader, I.L. Weissman, G.M. Wahl, Cancer stem cells — perspectives on current status and future directions: AACR workshop on cancer stem cells, Cancer Res. 66 (2006) 9339–9344.

[36] C.H. Park, D.E. Bergsagel, E.A. McCulloch, Mouse myeloma tumor stem cells: a primary cell culture assay, J. Natl. Cancer Inst. 46 (1971) 411–422.
[37] A.W. Hamburger, S.E. Salmon, Primary bioassay of human tumor stem cells, Science 197 (1977) 461–463.
[38] T. Reya, S.J. Morrison, M.F. Clarke, I.L. Weissman, Stem cells, cancer, and cancer stem cells, Nature 414 (2001) 105–111.
[39] D. Bonnet, J.E. Dick, Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell, Nat. Med. 3 (1997) 730–737.
[40] M. Al-Hajj, M.S. Wicha, A. Benito-Hernandez, S.J. Morrison, M.F. Clarke, Prospective identification of tumorigenic breast cancer cells, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 3983–3988.
[41] S.K. Singh, C. Hawkins, I.D. Clarke, J.A. Squire, J. Bayani, T. Hide, R.M. Henkelman, M.D. Cusimano, P.B. Dirks, Identification of human brain tumour initiating cells, Nature 432 (2004) 396–401.
[42] M.D. Taylor, H. Poppleton, C. Fuller, X. Su, Y. Liu, P. Jensen, S. Magdaleno, J. Dalton, C. Calabrese, J. Board, T. Macdonald, J. Rutka, A. Guha, A. Gajjar, T. Curran, R.J. Gilbertson, Radial glia cells are candidate stem cells of ependymoma, Cancer Cell 8 (2005) 323–335.
[43] C.A. O'Brien, A. Pollett, S. Gallinger, J.E. Dick, A human colon cancer cell capable of initiating tumour growth in immunodeficient mice, Nature 445 (2007) 106–110.
[44] L. Ricci-Vitiani, D.G. Lombardi, E. Pilozzi, M. Biffoni, M. Todaro, C. Peschle, R. De Maria, Identification and expansion of human colon-cancer-initiating cells, Nature 445 (2007) 111–115.
[45] K.J. Hope, L. Jin, J.E. Dick, Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity, Nat. Immunol. 5 (2004) 738–743.
[46] C. Li, D.G. Heidt, P. Dalerba, C.F. Burant, L. Zhang, V. Adsay, M. Wicha, M.F. Clarke, D.M. Simeone, Identification of pancreatic cancer stem cells, Cancer Res. 67 (2007) 1030–1037.
[47] M.E. Prince, R. Sivanandan, A. Kaczorowski, G.T. Wolf, M.J. Kaplan, P. Dalerba, I.L. Weissman, M.F. Clarke, L.E. Ailles, Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 973–978.
[48] D. Hanahan, R.A. Weinberg, Hallmarks of cancer: the next generation, Cell 144 (2011) 646–674.
[49] M. Dean, T. Fojo, S. Bates, Tumour stem cells and drug resistance, Nat. Rev. Cancer 5 (2005) 275–284.
[50] W.C. Dempke, V. Heinemann, Resistance to EGF-R (erbB-1) and VEGF-R modulating agents, Eur. J. Cancer 45 (2009) 1117–1128.
[51] L.V. Sequist, R.G. Martins, D. Spigel, S.M. Grunberg, A. Spira, P.A. Jänne, V.A. Joshi, D. McCollum, T.L. Evans, A. Muzikansky, G.L. Kuhlmann, M. Han, J.S. Goldberg, J. Settleman, A.J. Iafrate, J.A. Engelman, D.A. Haber, B.E. Johnson, T.J. Lynch, First-line gefitinib in patients with advanced non-small-cell lung cancer harboring somatic EGFR mutations, J. Clin. Oncol. 26 (2008) 2442–2449.
[52] T.J. Lynch, D.W. Bell, R. Sordella, S. Gurubhagavatula, R.A. Okimoto, B.W. Brannigan, P.L. Harris, S.M. Haserlat, J.G. Supko, F.G. Haluska, D.N. Louis, D.C. Christiani, J. Settleman, D.A. Haber, Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib, N. Engl. J. Med. 350 (2004) 2129–2139.
[53] W. Pao, V. Miller, M. Zakowski, J. Doherty, K. Politi, I. Sarkaria, B. Singh, R. Heelan, V. Rusch, L. Fulton, E. Mardis, D. Kupfer, R. Wilson, M. Kris, H. Varmus, EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 13306–13311.
[54] J.G. Paez, P.A. Jänne, J.C. Lee, S. Tracy, H. Greulich, S. Gabriel, P. Herman, F.J. Kaye, N. Lindeman, T.J. Boggon, K. Naoki, H. Sasaki, Y. Fujii, M.J. Eck, W.R. Sellers, B.E. Johnson, M. Meyerson, EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy, Science 304 (2004) 1497–1500.
[55] T. Fukui, T. Mitsudomi, Mutations in the epidermal growth factor receptor gene and effects of EGFR-tyrosine kinase inhibitors on lung cancers, Gen. Thorac. Cardiovasc. Surg. 56 (2008) 97–103.
[56] W. Pao, V.A. Miller, Epidermal growth factor receptor mutations, small-molecule kinase inhibitors, and non-small-cell lung cancer: current knowledge and future directions, J. Clin. Oncol. 23 (2005) 2556–2568.
[57] S. Kobayashi, T.J. Boggon, T. Dayaram, P.A. Jänne, O. Kocher, M. Meyerson, B.E. Johnson, M.J. Eck, D.G. Tenen, B. Halmos, EGFR mutation and resistance of non-small-cell lung cancer to gefitinib, N. Engl. J. Med. 352 (2005) 786–792.
[58] W. Pao, V.A. Miller, K.A. Politi, G.J. Riely, R. Somwar, M.F. Zakowski, M.G. Kris, H. Varmus, Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain, PLoS Med. 2 (2005) e73.
[59] C. Montagut, A. Dalmases, B. Bellosillo, M. Crespo, S. Pairet, M. Iglesias, M. Salido, M. Gallen, S. Marsters, S.P. Tsai, A. Minoche, S. Seshagiri, S. Somasekar, S. Serrano, H. Himmelbauer, J. Bellmunt, A. Rovira, J. Settleman, F. Bosch, J. Albanell, Identification of a mutation in the extracellular domain of the epidermal growth factor receptor conferring cetuximab resistance in colorectal cancer, Nat. Med. 18 (2012) 221–223.
[60] J.Y. Ljubimova, J. Portilla-Arias, R. Patil, H. Ding, S. Inoue, J.L. Markman, A. Rekechenetskiy, B. Konda, P.R. Gangalum, A. Chesnokova, A.V. Ljubimov, K.L. Black, E. Holler, Toxicity and efficacy evaluation of multiple targeted polymalic acid conjugates for triple negative breast cancer treatment, J. Drug Target. (2013)(in press).
[61] O. Trédan, C.M. Galmarini, K. Patel, I.F. Tannock, Drug resistance and the solid tumor microenvironment, J. Natl. Cancer Inst. 99 (2007) 1441–1454.
[62] L.A. Hazlehurst, W.S. Dalton, Mechanisms associated with cell adhesion mediated drug resistance (CAM-DR) in hematopoietic malignancies, Cancer Metastasis Rev. 20 (2001) 43–50.
[63] V.M. Weaver, S. Lelièvre, J.N. Lakins, M.A. Chrenek, J.C. Jones, F. Giancotti, Z. Werb, M.J. Bissell, β4 integrin-dependent formation of polarized three-dimensional architecture confers resistance to apoptosis in normal and malignant mammary epithelium, Cancer Cell 2 (2002) 205–216.
[64] D.S. Cowan, I.F. Tannock, Factors that influence the penetration of methotrexate through solid tissue, Int. J. Cancer 91 (2001) 120–125.
[65] N. Raghunand, B.P. Mahoney, R.J. Gillies, Tumor acidity, ion trapping and chemotherapeutics. II. pH-dependent partition coefficients predict importance of ion trapping on pharmacokinetics of weakly basic chemotherapeutic agents, Biochem. Pharmacol. 66 (2003) 1219–1229.
[66] B.P. Mahoney, N. Raghunand, B. Baggett, R.J. Gillies, Tumor acidity, ion trapping and chemotherapeutics. I. Acid pH affects the distribution of chemotherapeutic agents in vitro, Biochem. Pharmacol. 66 (2003) 1207–1218.
[67] CeL Davies, D.A. Berk, A. Pluen, R.K. Jain, Comparison of IgG diffusion and extracellular matrix composition in rhabdomyosarcomas grown in mice versus in vitro as spheroids reveals the role of host stromal cells, Br. J. Cancer 86 (2002) 1639–1644.
[68] P.A. Netti, D.A. Berk, M.A. Swartz, A.J. Grodzinsky, R.K. Jain, Role of extracellular matrix assembly in interstitial transport in solid tumors, Cancer Res. 60 (2000) 2497–2503.
[69] S. Vinogradov, X. Wei, Cancer stem cells and drug resistance: the potential of nanomedicine, Nanomedicine (Lond.) 7 (2012) 597–615.
[70] T.P. Padera, B.R. Stoll, J.B. Tooredman, D. Capen, E. di Tomaso, R.K. Jain, Pathology: cancer cells compress intratumour vessels, Nature 427 (2004) 695.
[71] K.M. Comerford, T.J. Wallace, J. Karhausen, N.A. Louis, M.C. Montalto, S.P. Colgan, Hypoxia-inducible factor-1-dependent regulation of the multidrug resistance (MDR1) gene, Cancer Res. 62 (2002) 3387–3394.
[72] G.L. Wang, G.L. Semenza, Purification and characterization of hypoxia-inducible factor 1, J. Biol. Chem. 270 (1995) 1230–1237.
[73] G.L. Semenza, Hypoxia, clonal selection, and the role of HIF-1 in tumor progression, Crit. Rev. Biochem. Mol. Biol. 35 (2000) 71–103.
[74] L. Milane, S. Ganesh, S. Shah, Z.F. Duan, M. Amiji, Multi-modal strategies for overcoming tumor drug resistance: hypoxia, the Warburg effect, stem cells, and multifunctional nanotechnology, J. Control. Release 155 (2011) 237–247.
[75] A.S. Chung, X. Wu, G. Zhuang, H. Ngu, I. Kasman, J. Zhang, J.M. Vernes, Z. Jiang, Y.G. Meng, F.V. Peale, W. Ouyang, N. Ferrara, An interleukin-17-mediated paracrine network promotes tumor resistance to anti-angiogenic therapy, Nat. Med. 19 (2013) 1114–1123.
[76] H. Ding, S. Inoue, A.V. Ljubimov, R. Patil, J. Portilla-Arias, J. Hu, B. Konda, K.A. Wawrowsky, M. Fujita, N. Karabalin, T. Sasaki, K.L. Black, E. Holler, J.Y. Ljubimova, Inhibition of brain tumor growth by intravenous poly (β-L-malic acid) nanobioconjugate with ph-dependent drug release [corrected], Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 18143–18148.
[77] Y. Huang, S. Goel, D.G. Duda, D. Fukumura, R.K. Jain, Vascular normalization as an emerging strategy to enhance cancer immunotherapy, Cancer Res. 73 (2013) 2943–2948.
[78] M. Wang, M. Thanou, Targeting nanoparticles to cancer, Pharmacol. Res. 62 (2010) 90–99.
[79] U. Prabhakar, H. Maeda, R.K. Jain, E.M. Sevick-Muraca, W. Zamboni, O.C. Farokhzad, S.T. Barry, A. Gabizon, P. Grodzinski, D.C. Blakey, Challenges and key considerations of the enhanced permeability and retention effect for nanomedicine drug delivery in oncology, Cancer Res. 73 (2013) 2412–2417.
[80] J.M. Koziara, T.R. Whisman, M.T. Tseng, R.J. Mumper, In-vivo efficacy of novel paclitaxel nanoparticles in paclitaxel-resistant human colorectal tumors, J. Control. Release 112 (2006) 312–319.
[81] M.A. Rouf, I. Vural, J.M. Renoir, A.A. Hincal, Development and characterization of liposomal formulations for rapamycin delivery and investigation of their antiproliferative effect on MCF7 cells, J. Liposome Res. 19 (2009) 322–331.
[82] A. Gabizon, R. Catane, B. Uziely, B. Kaufman, T. Safra, R. Cohen, F. Martin, A. Huang, Y. Barenholz, Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes, Cancer Res. 54 (1994) 987–992.
[83] Y. Zhang, H. Zhang, X. Wang, J. Wang, X. Zhang, Q. Zhang, The eradication of breast cancer and cancer stem cells using octreotide modified paclitaxel active targeting micelles and salinomycin passive targeting micelles, Biomaterials 33 (2012) 679–691.
[84] C. Gottstein, G. Wu, B.J. Wong, J.A. Zasadzinski, Precise quantification of nanoparticle internalization, ACS Nano 7 (6) (2013) 4933–4945.
[85] S.E. Krown, D.W. Northfelt, D. Osoba, J.S. Stewart, Use of liposomal anthracyclines in Kaposi's sarcoma, Semin. Oncol. 31 (2004) 36–52.
[86] P.G. Rose, Pegylated liposomal doxorubicin: optimizing the dosing schedule in ovarian cancer, Oncologist 10 (2005) 205–214.
[87] P. Sonneveld, R. Hajek, A. Nagler, A. Spencer, J. Bladé, T. Robak, S.H. Zhuang, J.L. Harousseau, R.Z. Orlowski, DOXIL-MMY-3001 Study Investigators, Combined pegylated liposomal doxorubicin and bortezomib is highly effective in patients with recurrent or refractory multiple myeloma who received prior thalidomide/lenalidomide therapy, Cancer 112 (2008) 1529–1537.
[88] R.P. Collea, F.W. Kruter, J.E. Cantrell, T.K. George, S. Kruger, A.M. Favret, D.L. Lindquist, A.M. Melnyk, R.E. Pluenneke, S.H. Shao, M.W. Crockett, L. Asmar, J. O'Shaughnessy, Pegylated liposomal doxorubicin plus carboplatin in patients with metastatic breast cancer: a phase II study, Ann. Oncol. 23 (2012) 2599–2605.
[89] M. Montanari, F. Fabbri, E. Rondini, G.L. Frassineti, R. Mattioli, S. Carloni, E. Scarpi, W. Zoli, D. Amadori, G. Cruciani, Phase II trial of non-pegylated liposomal doxorubicin and low-dose prednisone in second-line chemotherapy for hormone-refractory prostate cancer, Tumori 98 (2012) 696–701.
[90] R. Wang, R. Xiao, Z. Zeng, L. Xu, J. Wang, Application of poly(ethylene glycol)-distearoylphosphatidylethanolamine (PEG-DSPE) block copolymers and

their derivatives as nanomaterials in drug delivery, Int. J. Nanomedicine 7 (2012) 4185–4198.
[91] M.R. Vijayakumar, M.S. Muthu, S. Singh, Copolymers of poly(lactic acid) and D-α-tocopheryl polyethylene glycol 1000 succinate-based nanomedicines: versatile multifunctional platforms for cancer diagnosis and therapy, Expert Opin. Drug Deliv. 10 (2013) 529–543.
[92] L.M. Kaminskas, C.J. Porter, Targeting the lymphatics using dendritic polymers (dendrimers), Adv. Drug Deliv. Rev. 63 (2011) 890–900.
[93] O. Taratula, O. Garbuzenko, R. Savla, Y.A. Wang, H. He, T. Minko, Multifunctional nanomedicine platform for cancer specific delivery of siRNA by superparamagnetic iron oxide nanoparticles-dendrimer complexes, Curr. Drug Deliv. 8 (2011) 59–69.
[94] K. Knop, R. Hoogenboom, D. Fischer, U.S. Schubert, Poly(ethylene glycol) in drug delivery: pros and cons as well as potential alternatives, Angew. Chem. Int. Ed. Engl. 49 (2010) 6288–6308.
[95] G.A. Leget, M.S. Czuczman, Use of rituximab, the new FDA-approved antibody, Curr. Opin. Oncol. 10 (1998) 548–551.
[96] T.L. Brenner, V.R. Adams, First MAb approved for treatment of metastatic breast cancer, J. Am. Pharm. Assoc. (Wash) 39 (1999) 236–238.
[97] L. Goodman, Persistence–luck–avastin, J. Clin. Invest. 113 (2004) 934.
[98] Y. Humblet, Cetuximab: an IgG(1) monoclonal antibody for the treatment of epidermal growth factor receptor-expressing tumours, Expert Opin. Pharmacother. 5 (2004) 1621–1633.
[99] P. Zhang, L. Hu, Q. Yin, L. Feng, Y. Li, Transferrin-modified c[RGDfK]-paclitaxel loaded hybrid micelle for sequential blood–brain barrier penetration and glioma targeting therapy, Mol. Pharm. 9 (2012) 1590–1598.
[100] W. Wang, F. Zhou, L. Ge, X. Liu, F. Kong, Transferrin-PEG-PE modified dexamethasone conjugated cationic lipid carrier mediated gene delivery system for tumor-targeted transfection, Int. J. Nanomedicine 7 (2012) 2513–2522.
[101] T.R. Daniels, E. Bernabeu, J.A. Rodríguez, S. Patel, M. Kozman, D.A. Chiappetta, E. Holler, J.Y. Ljubimova, G. Helguera, M.L. Penichet, The transferrin receptor and the targeted delivery of therapeutic agents against cancer, Biochim. Biophys. Acta 2012 (1820) 291–317.
[102] D. Peer, J.M. Karp, S. Hong, O.C. Farokhzad, R. Margalit, R. Langer, Nanocarriers as an emerging platform for cancer therapy, Nat. Nanotechnol. 2 (2007) 751–760.
[103] Z. Xiao, O.C. Farokhzad, Aptamer-functionalized nanoparticles for medical applications: challenges and opportunities, ACS Nano 6 (2012) 3670–3676.
[104] S.M. Weis, D.A. Cheresh, αV integrins in angiogenesis and cancer, Cold Spring Harb. Perspect. Med. 1 (2011) a006478.
[105] E. Ruoslahti, Peptides as targeting elements and tissue penetration devices for nanoparticles, Adv. Mater. 24 (2012) 3747–3756.
[106] V. Gregorc, G. Citterio, G. Vitali, A. Spreafico, P. Scifo, A. Borri, G. Donadoni, G. Rossoni, A. Corti, F. Caligaris-Cappio, A. Del Maschio, A. Esposito, F. De Cobelli, F. Dell'Acqua, A. Troysi, P. Bruzzi, A. Lambiase, C. Bordignon, Defining the optimal biological dose of NGR-hTNF, a selective vascular targeting agent, in advanced solid tumours, Eur. J. Cancer 46 (2010) 198–206.
[107] C. Pichon, L. Billiet, P. Midoux, Chemical vectors for gene delivery: uptake and intracellular trafficking, Curr. Opin. Biotechnol. 21 (2010) 640–645.
[108] F. Pittella, M. Zhang, Y. Lee, H.J. Kim, T. Tockary, K. Osada, T. Ishii, K. Miyata, N. Nishiyama, K. Kataoka, Enhanced endosomal escape of siRNA-incorporating hybrid nanoparticles from calcium phosphate and peg-block charge-conversional polymer for efficient gene knockdown with negligible cytotoxicity, Biomaterials 32 (2011) 3106–3114.
[109] E.S. Lee, Z. Gao, D. Kim, K. Park, I.C. Kwon, Y.H. Bae, Super pH-sensitive multifunctional polymeric micelle for tumor pH(e) specific TAT exposure and multidrug resistance, J. Control. Release 129 (2008) 228–236.
[110] M. Mohri, H. Nitta, J. Yamashita, Expression of multidrug resistance-associated protein (MRP) in human gliomas, J. Neurooncol. 49 (2000) 105–115.
[111] R. Patil, J. Portilla-Arias, H. Ding, S. Inoue, B. Konda, J. Hu, K.A. Wawrowsky, P.K. Shin, K.L. Black, E. Holler, J.Y. Ljubimova, Temozolomide delivery to tumor cells by a multifunctional nano vehicle based on poly(β-L-malic acid), Pharm. Res. 27 (2010) 2317–2329.
[112] R. Patil, J. Portilla-Arias, H. Ding, B. Konda, A. Rekechenetskiy, S. Inoue, K.L. Black, E. Holler, J.Y. Ljubimova, Cellular delivery of doxorubicin via pH-controlled hydrazone linkage using multifunctional nano vehicle based on poly(β-L-malic acid), Int. J. Mol. Sci. 13 (2012) 11681–11693.
[113] H. Yuan, X. Li, J. Wu, J. Li, X. Qu, W. Xu, W. Tang, Strategies to overcome or circumvent P-glycoprotein mediated multidrug resistance, Curr. Med. Chem. 15 (2008) 470–476.
[114] J. Wu, Y. Lu, A. Lee, X. Pan, X. Yang, X. Zhao, R.J. Lee, Reversal of multidrug resistance by transferrin-conjugated liposomes co-encapsulating doxorubicin and verapamil, J. Pharm. Pharm. Sci. 10 (2007) 350–357.
[115] S. Ganta, M. Amiji, Coadministration of paclitaxel and curcumin in nanoemulsion formulations to overcome multidrug resistance in tumor cells, Mol. Pharm. 6 (2009) 928–939.
[116] M. Saad, O.B. Garbuzenko, T. Minko, Co-delivery of siRNA and an anticancer drug for treatment of multidrug-resistant cancer, Nanomedicine (Lond.) 3 (2008) 761–776.
[117] E. Bajelan, A. Haeri, A.M. Vali, S.N. Ostad, S. Dadashzadeh, Co-delivery of doxorubicin and PSC 833 (Valspodar) by stealth nanoliposomes for efficient overcoming of multidrug resistance, J. Pharm. Pharm. Sci. 15 (2012) 568–582.
[118] K. Wang, X. Wu, J. Wang, J. Huang, Cancer stem cell theory: therapeutic implications for nanomedicine, Int. J. Nanomedicine 8 (2013) 899–908.
[119] Q.L. Tang, Z.Q. Zhao, J.C. Li, Y. Liang, J.Q. Yin, C.Y. Zou, X.B. Xie, Y.X. Zeng, J.N. Shen, T. Kang, J. Wang, Salinomycin inhibits osteosarcoma by targeting its tumor stem cells, Cancer Lett. 311 (2011) 113–121.
[120] A. Verma, S. Guha, P. Diagaradjane, A.B. Kunnumakkara, A.M. Sanguino, G. Lopez-Berestein, A.K. Sood, B.B. Aggarwal, S. Krishnan, J.G. Gelovani, K. Mehta, Therapeutic significance of elevated tissue transglutaminase expression in pancreatic cancer, Clin. Cancer Res. 14 (2008) 2476–2483.
[121] J.E. Visvader, G.J. Lindeman, Cancer stem cells in solid tumours: accumulating evidence and unresolved questions, Nat. Rev. Cancer 8 (2008) 755–768.
[122] J.A. Burger, A. Peled, CXCR4 antagonists: targeting the microenvironment in leukemia and other cancers, Leukemia 23 (2009) 43–52.
[123] J. Prakash, E. de Jong, E. Post, A.S. Gouw, L. Beljaars, K. Poelstra, A novel approach to deliver anticancer drugs to key cell types in tumors using a PDGF receptor-binding cyclic peptide containing carrier, J. Control. Release 145 (2010) 91–101.
[124] M. Goldsmith, S. Mizrahy, D. Peer, Grand challenges in modulating the immune response with RNAi nanomedicines, Nanomedicine (Lond.) 6 (2011) 1771–1785.
[125] H. Poeck, R. Besch, C. Maihoefer, M. Renn, D. Tormo, S.S. Morskaya, S. Kirschnek, E. Gaffal, J. Landsberg, J. Hellmuth, A. Schmidt, D. Anz, M. Bscheider, T. Schwerd, C. Berking, C. Bourquin, U. Kalinke, E. Kremmer, H. Kato, S. Akira, R. Meyers, G. Häcker, M. Neuenhahn, D. Busch, J. Ruland, S. Rothenfusser, M. Prinz, V. Hornung, S. Endres, T. Tüting, G. Hartmann, 5′-triphosphate-siRNA: turning gene silencing and Rig-I activation against melanoma, Nat. Med. 14 (2008) 1256–1263.
[126] M. Kortylewski, P. Swiderski, A. Herrmann, L. Wang, C. Kowolik, M. Kujawski, H. Lee, A. Scuto, Y. Liu, C. Yang, J. Deng, H.S. Soifer, A. Raubitschek, S. Forman, J.J. Rossi, D.M. Pardoll, R. Jove, H. Yu, In vivo delivery of siRNA to immune cells by conjugation to a TLR9 agonist enhances antitumor immune responses, Nat. Biotechnol. 27 (2009) 925–932.
[127] N. Khairuddin, M.P. Gantier, S.J. Blake, S.Y. Wu, M.A. Behlke, B.R. Williams, N.A. McMillan, siRNA-induced immunostimulation through TLR7 promotes antitumoral activity against HPV-driven tumors in vivo, Immunol. Cell Biol. 90 (2012) 187–196.
[128] H. Ding, G. Helguera, J.A. Rodríguez, J. Markman, R. Luria-Pérez, P. Gangalum, J. Portilla-Arias, S. Inoue, T.R. Daniels-Wells, K. Black, E. Holler, M.L. Penichet, J.Y. Ljubimova, Polymalic acid nanobioconjugate for simultaneous inhibition of tumor growth and immunostimulation in HER2/*neu*-positive breast cancer, J. Control. Release 171 (3) (2013 Nov 10) 322–329.
[129] H. Yu, Z. Xu, X. Chen, L. Xu, Q. Yin, Z. Zhang, Y. Li, Reversal of lung cancer multidrug resistance by pH-responsive micelleplexes mediating co-delivery of siRNA and paclitaxel, Macromol. Biosci. (2013)(in press).
[130] L. Qian, J. Zheng, K. Wang, Y. Tang, X. Zhang, H. Zhang, F. Huang, Y. Pei, Y. Jiang, Cationic core-shell nanoparticles with carmustine contained within o(6)-benzylguanine shell for glioma therapy, Biomaterials 34 (2013) 8968–8978.
[131] P.Y. Chang, S.F. Peng, C.Y. Lee, C.C. Lu, S.C. Tsai, T.M. Shieh, T.S. Wu, M.G. Tu, M.Y. Chen, J.S. Yang, Curcumin-loaded nanoparticles induce apoptotic cell death through regulation of the function of MDR1 and reactive oxygen species in cisplatin-resistant CAR human oral cancer cells, Int. J. Oncol. 43 (2013) 1141–1150.
[132] A.R. Burke, R.N. Singh, D.L. Carroll, J.C. Wood, R.B. D'Agostino, P.M. Ajayan, F.M. Torti, S.V. Torti, The resistance of breast cancer stem cells to conventional hyperthermia and their sensitivity to nanoparticle-mediated photothermal therapy, Biomaterials 33 (2012) 2961–2970.
[133] S. Zhao, S. Tan, Y. Guo, J. Huang, M. Chu, H. Liu, Z. Zhang, Ph-sensitive docetaxel-loaded D-α-tocopheryl polyethylene glycol succinate-poly(β-amino ester) copolymer nanoparticles for overcoming multidrug resistance, Biomacromolecules 14 (2013) 2636–2646.
[134] S.K. Swaminathan, E. Roger, U. Toti, L. Niu, J.R. Ohlfest, J. Panyam, CD133-targeted paclitaxel delivery inhibits local tumor recurrence in a mouse model of breast cancer, J. Control. Release 171 (3) (2013 Nov 10) 280–287.
[135] J. Zhou, T.R. Patel, R.W. Sirianni, G. Strohbehn, M.Q. Zheng, N. Duong, T. Schafbauer, A.J. Huttner, Y. Huang, R.E. Carson, Y. Zhang, D.J. Sullivan, J.M. Piepmeier, W.M. Saltzman, Highly penetrative, drug-loaded nanocarriers improve treatment of glioblastoma, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 11751–11756.
[136] X. Zhang, H. Yang, K. Gu, J. Chen, M. Rui, G.L. Jiang, In vitro and in vivo study of a nanoliposomal cisplatin as a radiosensitizer, Int. J. Nanomedicine 6 (2011) 437–444.
[137] Q. Yin, J. Shen, Z. Zhang, H. Yu, L. Chen, W. Gu, Y. Li, Multifunctional nanoparticles improve therapeutic effect for breast cancer by simultaneously antagonizing multiple mechanisms of multidrug resistance, Biomacromolecules 14 (2013) 2242–2252.
[138] C. Yang, F. Xiong, J. Wang, J. Dou, J. Chen, D. Chen, Y. Zhang, S. Luo, N. Gu, Anti-ABCG2 monoclonal antibody in combination with paclitaxel nanoparticles against cancer stem-like cell activity in multiple myeloma, Nanomedicine (Lond.) (2013)(in press).
[139] B. Perlstein, S.A. Finniss, C. Miller, H. Okhrimenko, G. Kazimirsky, S. Cazacu, H.K. Lee, N. Lemke, S. Brodie, F. Umansky, S.A. Rempel, M. Rosenblum, T. Mikkelsen, S. Margel, C. Brodie, TRAIL conjugated to nanoparticles exhibits increased anti-tumor activities in glioma cells and glioma stem cells in vitro and in vivo, Neuro Oncol. 15 (2013) 29–40.
[140] D. Wang, J. Tang, Y. Wang, S. Ramishetti, Q. Fu, K. Racette, F. Liu, Multifunctional nanoparticles based on a single-molecule modification for the treatment of drug-resistant cancer, Mol. Pharm. 10 (2013) 1465–1469.
[141] S. Gaca, S. Reichert, C. Rödel, F. Rödel, J. Kreuter, Survivin-miRNA-loaded nanoparticles as auxiliary tools for radiation therapy: preparation, characterisation, drug release, cytotoxicity and therapeutic effect on colorectal cancer cells, J. Microencapsul. 29 (2012) 685–694.
[142] S. Kato, M. Kimura, K. Kageyama, H. Tanaka, N. Miwa, Enhanced radiosensitization by liposome-encapsulated pimonidazole for anticancer effects on human melanoma cells, J. Nanosci. Nanotechnol. 12 (2012) 4472–4477.
[143] E.C. Dreaden, B.E. Gryder, L.A. Austin, B.A. Tene Defo, S.C. Hayden, M. Pi, L.D. Quarles, A.K. Oyelere, M.A. El-Sayed, Antiandrogen gold nanoparticles dual-target and overcome treatment resistance in hormone-insensitive prostate cancer cells, Bioconjug. Chem. 23 (2012) 1507–1512.

[144] C. Yang, J. Wang, D. Chen, J. Chen, F. Xiong, H. Zhang, Y. Zhang, N. Gu, J. Dou, Paclitaxel-Fe_3O_4 nanoparticles inhibit growth of CD138(−) CD34(−) tumor stem-like cells in multiple myeloma-bearing mice, Int. J. Nanomedicine 8 (2013) 1439–1449.
[145] H.S. Günther, N.O. Schmidt, H.S. Phillips, D. Kemming, S. Kharbanda, R. Soriano, Z. Modrusan, H. Meissner, M. Westphal, K. Lamszus, Glioblastoma-derived stem cell-enriched cultures form distinct subgroups according to molecular and phenotypic criteria, Oncogene 27 (2008) 2897–2909.
[146] N. Borsellino, A. Belldegrun, B. Bonavida, Endogenous interleukin 6 is a resistance factor for cis-diamminedichloroplatinum and etoposide-mediated cytotoxicity of human prostate carcinoma cell lines, Cancer Res. 55 (1995) 4633–4639.
[147] P.B. Gupta, T.T. Onder, G. Jiang, K. Tao, C. Kuperwasser, R.A. Weinberg, E.S. Lander, Identification of selective inhibitors of cancer stem cells by high-throughput screening, Cell 138 (2009) 645–659.
[148] R. Siegel, D. Naishadham, A. Jemal, Cancer statistics, 2013, CA Cancer J. Clin. 63 (2013) 11–30.