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Advances in mesoporous silica-based nanocarriers for co-delivery and combination therapy against cancer

Rafael R. Castillo\textsuperscript{a,b}, Montserrat Colilla\textsuperscript{a,b} and María Vallet-Regí\textsuperscript{a,b}

ABSTRACT

1. Introduction

Currently, one of the pivotal pillars of cancer therapy is based on the use of chemical compounds to induce cellular death. However, the systemic administration of cytotoxic drugs enhances cell death in both tumor and healthy tissues. Unluckily this lack of selectivity usually provokes severe side effects in the patient that reduce its potential applicability. To avoid this limitation, the biomedical scientific community has done many efforts to increase the therapeutic profiles of those cytotoxic drugs. One of the most promising approaches is based on the development of nanocarriers to selectively reach tumor areas and release on-site cytotoxic payloads \cite{1,2}. Unfortunately, the extensive use of chemotherapy has also led to additional problems that remain unsolved. One of the most important is the acquired resistance of surviving cells against antiproliferative drugs because of not enough evidences of safety and therapeutic efficacy of these nanosystems \cite{12}.

This review overviews the scientific efforts developed up to date to provide MSNs-based nanosystems of enhanced dual therapeutic actions against MDR in cancer cells \cite{15–19}. This challenging goal may be tackled through three main approaches: (i) improving the nanosystems’ targeting toward diseased cells by enhancing both recognition and specificity; (ii) enhancing the nanosystems’ apoptotic effect throughout codelivery of several therapeutic agents; and (iii) improving killing capability by combination of drug delivery with apoptosis induced by physical methods.

2. Enhancing the recognition by dual targeting

Most of reported applications of nanocarriers, including those approved as pharmaceuticals, are mainly based on unspecific passive accumulation within the tumor mass due to the well-known enhanced permeation and retention (EPR) effect \cite{20,21}. Recent investigations have demonstrated that the selectivity can be increased by active targeting, i.e. surface decoration of nanocarrier with targeting ligands that are able to promote internalization through recognition of diseased cells overexpressing specific receptors. Thus, active targeting provides better therapeutic profiles as nanocarriers are preferentially accumulated by
Another interesting approach is based on the maximization of the selectivity of nanocarriers to deal with MDR tumor cells. Furthermore, a highly promising alternative to increase cell death will be overviewed, focusing on either membrane systems or drug efflux pumps that create this resistance to antitumor drugs.

One recent attractive strategy to overcome the high survival ratio of nanocarriers and among them mesoporous silica nanoparticles is devoted to the efforts accomplished so far to increase the efficiency of MSNs-based nanomedicines for cancer therapy. Among known factors of enhanced survival there could be found antiapoptotic routes or drug efflux pumps that create this resistance, like raw drug administration. In spite of guidance has improved the selectivity and efficiency of the treatment towards diseased cells, there is still an issue when multidrug resistance appears.

Another interesting approach is based on the maximization of the killing potential of carriers by including combinations of substances as cargoes able to exert simultaneous or synergistic action on more than one critical metabolic pathway. Furthermore, a highly promising alternative to increase cell death could be also generated by dual combination of antiproliferative drugs with physical stimuli able to trigger additional apoptotic responses.

This box summarizes key points contained in the article.

Target cells. Nevertheless, the uptake of nanocarriers by other nondiseased cells in which the implicated receptors are also present is still a challenging problem. In an effort to overcome these issues, some advanced targeting approaches have been developed [22,23]. In the next sections we will focus on MSNs as versatile and modular systems, albeit some of the described strategies are also feasible for other nanocarriers [24]. Some relevant examples of liposomal and polymeric formulations can be found by the reader in the Expert Opinion section.

Unspecific cellular recognition may produce important and undesired side effects, similar to those generated by conventional chemotherapy. Firstly, a poor specificity in cellular recognition may mismatch the targeting destination from the therapeutic area, thus lowering the treatment efficiency. And secondly, but also important, is that non- or poorly-targeted systems might lead in long-term, low-dose exposure of diseased cells to cytotoxic drugs, which could increase the chances of developing MDR by those tumors. This can take place either by evolution through down-regulation of implicated receptors, or by overexpression of efflux pumps such as P-glycoprotein (Pgp) [25–27], and/or other ATP-binding cassettes (ABC) transporters responsible of xenobiotics draining out of the cell [28,29].

In this section, some current strategies aimed at improving the efficiency of MSNs-based nanomedicines for cancer therapy will be overviewed, focusing on either membrane–nuclear targeting or vascular–cellular targeting. Special emphasis will be devoted to the efforts accomplished so far to increase the selectivity of nanocarriers to deal with MDR tumor cells.

2.1. Membrane–nuclear targeting

As previously stated, one of the main challenges in the application of nanomedicine to cancer treatment is the specific delivery of nanocarriers to tumors. In this sense, the use of highly expensive but specific antibodies would partially solve this problem, as they could specifically recognize diseased cells, although they may originate immunogenic responses in the organism. They also offer important advantages since antibodies are the most specific targeting moieties known up to date. Thus an antibody targeted carrier would be expected to mainly interact with the target cells and therefore minimize the misplaced release of chemotherapeutics. However, many of the known antibodies target alike diseased and healthy cells that express the complementary antigen, which could discard them for cell targeted cancer therapy. In addition the use of antibodies is problematic as its grafting onto nanoparticles may require harsh conditions which may lead to their denaturalization. For this reason, new vectorization strategies are worth of exploring, since efficient deliveries of chemotherapeutic could significantly reduce the side effects associated to the inherent drug toxicity and thus the development of drug resistance.

In one the first reported examples, Pan et al. designed doxorubicin (DOX) loaded MSNs decorated with two different bioactive peptides on the surface; one responsible of cellular membrane recognition and another able to actively deliver the carrier to the nucleus (Figure 1(a)) [30]. The chosen c(RGDyC) peptide interacted with the αvβ3-integrin present on cellular membranes of HeLa cellular line, while the transactivator of transcription (TAT) peptide from human immunodeficiency virus (HIV) facilitated the penetration to the nucleus of the cell [31–33]. Their results evidenced a preferential accumulation of dual-targeted MSNs compared with nontargeted or single-TAT targeted nanoparticles (Entry 1, Table 1).

In a similar way, Xiong et al. designed a double-targeted system based on two small bioactive molecules: folic acid (FA), able to interact with the folate receptors (a-FR) overexpressed on HeLa cell membranes, and dexamethasone (DEX) which interacts with the nuclear glucocorticoid receptors able to induce nuclear translocation [34]. The use of DEX decorated nanoparticles showed a fivefold increase in nuclear location as confirmed by flow cytometry analyses (Entry 2, Table 1).

2.2. Vascular–cellular targeting

It is known that tumor growth clearly depends on supplies received, so usually the result is the development of highly vascularized areas induced by angiogenic substances produced by those tumor cells. Thus antiangiogenesis became an interesting approach to the increase of therapeutic profile of nanomedicines as the targeting of tumor new-forming vessels that could locally inhibit tumor growth [36–38]. Although this may induce tumor invasion and metastasis due to nutrient outage, a proper combination with cellular proapoptotic substances could effectively decrease its aggressiveness.

Some combinations of angiogenic therapy together with strategies to enhance cellular uptake have been reported. The results indicate that the overall accumulation of cytotoxic drugs within the tumor is improved, which increases the efficiency of the treatment. In a recent paper by Qiao et al. hollow MSNs (HMSNs) were functionalized with a single...
heptapeptide (t-Lyp-1) to target simultaneously two proteins within the Neurophilin family (NRP1 involved in angiogenesis and NRP2 in lymphangiogenesis) to enhance penetration into tumor parenchyma and arrest tumor development (Entry 3, Table 1) [35].

3. Enhancing the cell-death effect by combination therapy

3.1. Dual delivery of cytotoxic drugs and small interfering RNAs (siRNAs)

As previously remarked, the development of strategies to overcome MDR tumors would constitute a great milestone in cancer therapy [39]. Although some advances have been achieved to circumvent this issue, their immediate application still remains unclear.

Usually MDR defines an ability of cancerous cells to become resistant to usual chemotherapeutics, which could be due to two independent processes, namely pump and nonpump resistance. The pump resistance mechanism is originated by overexpression of several ATP-dependent membrane proteins, such as Pgp [25,26,40], or other proteins responsible of active drug efflux from the cell. Thereby this continuous drug expelling reduces the overall amount of drug within the cell, thus decreasing its therapeutic efficacy. The Pgp is usually highly expressed in difficult-to-treat cancers such as stomach, breast and pancreas carcinomas; unfortunately it is also quite common in other metastatic tumors, and when highly expressed, it induces drug resistance through drug drainage efflux out of the cell. The nonpump resistance mechanisms induce the activation of antiapoptotic defense usually mediated by antiapoptotic proteins such as Bcl family from which the most representative is Bcl-2 (B-Cell Lymphoma 2) [41,42] or heat-shock proteins (HSP) [43], whose overexpression prevents cellular death.

As conventional chemotherapy usually activates both resistance processes, it would be necessary to inhibit those mechanisms to fight against MDR. However the current state of the art is mainly based in the disruption of one resistance paths, although promising results are obtained with the new generation targeted devices.

Small interfering RNAs or silencing RNAs (siRNAs) are a kind of nucleic acids able to interfere with the normal gene expression of mammalian cells by competing with messenger RNAs (mRNAs). This is originated because siRNA are able to potently, persistently and specifically disrupt the normal effect of mRNAs that encodes drug-resistance related proteins, thus increasing the efficacy of the drug-based treatment. MSNs based nanoparticles are of special interest because they can deliver cytotoxic and siRNA species simultaneously more efficiently than other nanosystem; and therefore increase the therapeutic profile by maximizing the concentration of chemotherapeutic while silencing the effect of proteins responsible of drug resistance [44,45].

For this strategy, it is important to note that the anchoring of siRNAs to the nanocarrier must be reversible as its way of action requires a final detachment of the nucleic acid to perform its therapeutic effect (Figure 1(b)). In this sense, MSNs are of great interest because their surface charge can be easily tailored to undergo electrostatic interaction with nucleic acids, oppositely to other nanoplatforms that require a complete redesign or specific synthesis to switch the superficial charge. Nevertheless there are many different examples on the literature where non-MSNs based nanoplatforms are also successfully employed. For a recent review on this topic please check reference [46].
In the first reported example on MSNs, Chen et al. functionalized the negatively charged outermost surface of DOX-loaded MSNs with positively charged generation 2(G2) amine terminated polyamidoamine (PAMAM) dendrimer via electrostatic attractive interactions that disappear under acidic lisosomal pH. The resulting cationic system was then suitable for the incorporation of the anionic siRNA effective against mRNA encoding Bcl-2 antiapoptotic protein [47]. Authors found that their complex increased cellular death in MDR A2780/AD human ovarian cancer cells, with an enhanced apoptotic effect 132 times greater than those of free DOX due to the suppression of Bcl-2 nonpump resistance. Moreover, the data demonstrated that DOX was mainly localized on the perinuclear region, what seemed to indicate an effective bypass of pump mediated resistance (Entry 1, Table 2). A similar strategy was employed by the group of Zhao et al., who used DOX-loaded HMSN in which the role of polycationic linking material was played by a polyethyleneimine (PEI) polymer [48]. In this work the authors also included FA as targeting moiety. The evaluation against positive (HeLa) and negative (MCF-7) α-FR breast cancer cells showed a clear internalization preference by the HeLa cell line. Subsequently, the down expression of Bcl-2 significantly reduced the viability of HeLa compared to that of MCF-7 cells (Entry 2, Table 2).

A similar design has also been reported for the delivery of Pgp siRNA and DOX in several breast cancer cell lines by Meng et al. [49,50]. As Pgp is one of the major pathways in drug resistance, the silencing of this efflux would increase the efficacy of the employed cytotoxic drug. In these works the authors chose phosphonate coated MSNs to guarantee high dispersability and biosafety. These nanosystems were able to electrostatically bind PEI polymer for further complexation of the siRNA, which exhibit an acidic dependent release. The treatment of DOX-resistant KB-V1 breast carcinoma cells with the DOX-siRNA system resets the drug concentration values to those showed by the sensitive cells. In the most recent article the authors evaluated the behavior of different reported siRNA such as pump dependent (Pgp, MRPI and ABCG2) and non-pump dependent (Bcl-2, cMYC and PXR) [50]. From all tested cases authors found DOX-Pgp siRNA as the best combination for drug resistance reversion (Entries 3 and 4, Table 2).

Apart from the examples involving Bcl-2 and Pgp siRNAs, there also has been reported reversal resistance employing the micro RNA 221 (miR221), which is involved in several pathways such as angiogenesis or cell migration. Neuronal cancers as glioma are usually treated with a DNA alkylating agent such as temozolomide (TMZ) but its effectiveness is sometimes comprised by drug resistance. However, the combination of anti-miR221 with TMZ has proven to reverse glioma resistances according to the results reported by De Cola’s group (Entry 5, Table 2) [51]. Like in previous examples the siRNA-MSNs binding relies on electrostatic interactions, excepting that in this case the negatively charged silica particle binds a peptide-nucleic acid conjugate in which a peptidic section bears the positive charge responsible for electrostatic interaction. *In vitro* studies with this dual system show an important synergistic action of resistance reversion in T98G glioma cells not reached with each therapeutic alone.

Another interesting approach is the co-delivery of a chemotherapeutic drug in combination with an angiogenesis down regulation RNA [36]. Such system, reported by Yin et al. combined the apoptotic effect of the drug with a nutrient outflow to the tumor [52]. In this case the authors evolved the system with TAT cell penetrating peptide to increase the effect of the chemotherapeutic while building an on demand pH sensitive drug-releasing mechanism. The evaluation of this system on human hepatocarcinoma provided an additional advantage, as no guidance was required as liver spontaneously accumulates most of xenobiotics. For the conjugation of both DOX and vascular endothelial growth factor (VEGF) siRNA the authors designed a multilayer system based on anionic and cationic layers able to sequentially disintegrate. The system releases stepwise the siRNA in the cytosol and the cytotoxic DOX in the perinuclear area due to the nuclear targeting of TAT peptide (Entry 6, Table 2) [31,32]. The potential therapeutic effect of anti VEGF siRNAs containing nanomedicines was also confirmed by Chen et al. [53]. Thus magnetic MSNs were functionalized with vasohibin-2 silencing RNA and the KALA fusogenic peptide, responsible of facilitating internalization in the outer region. Although authors did not use a conventional chemotherapeutic drug for apoptosis induction, the combined action of siRNA and the fusogenic peptide KALA proved to be effective in the *in vivo* treatment of ovarian adenocarcinoma tumors. Their system showed a sixfold inhibition on tumor growth, compared with normal

### Table 2. MSNs models employed for dual therapeutic actions based on gene silencing.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Delivery system</th>
<th>siRNA</th>
<th>Tumor targeting</th>
<th>Cytotoxic loaded</th>
<th>Cellular line</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MSNs</td>
<td>Bcl-2</td>
<td>None</td>
<td>DOX</td>
<td>A2780/AD (Ovarian)</td>
<td>[47]</td>
</tr>
<tr>
<td>2</td>
<td>Hollow MSNs</td>
<td>Bcl-2</td>
<td>Folic acid</td>
<td>DOX</td>
<td>HeLa (Folic+) (breast)</td>
<td>[48]</td>
</tr>
<tr>
<td>3</td>
<td>MSNs</td>
<td>p-Glycoprotein (Pgp)</td>
<td>None</td>
<td>DOX</td>
<td>KB-31 (DOX sensitive)</td>
<td>[49]</td>
</tr>
<tr>
<td>4</td>
<td>MSNs</td>
<td>Several siRNA (pump: Pgp, MRPI, ABCG2 nonpump: Bcl-2, cMYC, PXR)</td>
<td>None</td>
<td>DOX</td>
<td>KB-V1 (DOX resistant)</td>
<td>[50]</td>
</tr>
<tr>
<td>5</td>
<td>MSNs</td>
<td>Anti-miR221</td>
<td>None</td>
<td>TMZ</td>
<td>C6 (TMZ sensitive), T98G (TMZ resistant) (glioma)</td>
<td>[51]</td>
</tr>
<tr>
<td>6</td>
<td>MSNs</td>
<td>VEGF-siRNA</td>
<td>TAT peptide (nuclear)</td>
<td>DOX</td>
<td>GY-7703 (hepatic)</td>
<td>[52]</td>
</tr>
<tr>
<td>7</td>
<td>Magnetic MSNs</td>
<td>Vasohibin-2 (VEGF-siRNA)</td>
<td>KALA peptide (fusogenic)</td>
<td>None</td>
<td>SKOV3 (ovarian)</td>
<td>[53]</td>
</tr>
</tbody>
</table>

DOX: doxorubicin; TMZ: temozolomide; Bcl-2: B-cell lymphoma 2; VEGF: vascular endothelial growth factor; TAT: transactivator of transcription.
tumor progression. Unlike the previous combinations, only for therapeutic purposes, this system allows the diagnosis of tumor evolution by magnetic resonance imaging (Entry 7, Table 2).

### 3.2. Multiple cytotoxic drugs delivery

In the fight against tumor MDR, the combination of chemotherapeutic drugs is a promising strategy as the coadministration of more than one chemotherapeutic may hamper the cellular adaptation [54]. In fact this is the logical evolution of current therapies, which are based on the sequential administration of different drug cycles (or combined therapies) to attack the tumor on several fronts. A promising approach for enhancing cell sensitivity against chemotherapeutic compounds could be the delivery of hybrid drugs [55,56], i.e. those combinations of single independent pharmacophores in a single molecule. Although this strategy could pave the way to new generation drugs based on simultaneous delivery or dual-actions, this could not be suitable for all purposes. Some common issues for hybrid design are, firstly the nature of drugs that could not allow conjugation without compromising their activity; secondly, a misplacement of pharmacophore destination mainly when both components act on different places; and thirdly, a poor pharmacokinetic/dynamic profile of hybrids compared to its individual components. Thus, the development of nanosized based carriers with its inherent drawbacks could be used to deliver combinations of discrete drugs and contribute to the improvement of therapy against MDR tumors by complementing the pharmacological profile of those hybrid drugs with a different way of delivery.

The role of MSNs based nanomedicines against cancer is promising as they are known to preserve the loaded compounds from external degradation and, if properly functionalized, also prevent those compounds from premature clearance or allowing programmed delivery. However, when compared to other systems such as polymeric nanoparticles or liposomes, the coloading of several guests molecules into MSNs is complex, as it depends on many parameters such as the different solubility of guest molecules in the loading solvent, the different diffusion rates throughout the pores, the strength of interaction between loaded molecules and silica or the nondiscardable retention value on the outer organic layer present in many nanodevices, among others. Oppositely, the preparation of polymeric and liposomal formulations with two or more loaded compounds is easier as usually loading and formation step occurs simultaneously. Regarding liposomal nanocarriers, their preparation is usually accomplished by adding the liposome components into a suspension of drug(s) under vigorous stirring. This forms the corresponding carriers containing the guest molecule(s) usually maintaining the same ratio employed previously; about polymeric carriers the common nanoprecipitation technique employs a solution of both drug(s) and polymer which is carefully precipitated onto a second solvent, again under vigorous stirring, which produces the simultaneous precipitation of polymer and drug(s) that are maintained within the three-dimensional polymeric matrix. Because of this, it is easier to find more literature based on polymeric or liposomal carriers than available for MSNs. Within this line, different nonsilica based nanocarriers loaded with different combinations of cytotoxic drugs, have been recently reviewed by Gadde [57].

However nonsilica based systems also suffer important drawbacks that limit their use as nanotherapeutics. For example, there are postfunctionalization difficulties for soft nanocarriers originated by their low stability when organic solvents are present or against many cycles of isolation. Thus, although multidrug loading of MSNs is complex, they still present advantages against other common nanocarriers. Along this section there will be overviewed the efforts made in multidrug loading of MSNs toward MDR cancer therapy. As indicated, the loading and release of compounds from the MSNs’ mesopores is not an easy task, nevertheless an adequate delivery could be achieved when effective coatings are used. Their role is to hamper the undesired premature release of loaded compounds. This concept was first introduced to control the dual release of two molecules from MSNs [58]. As there will be discussed along the next paragraphs and summarized in Table 3, other strategies have been developed to attain this goal.

Although most efforts have been focused on the development and use of coatings, there are also some examples in which one of the delivered drugs acts as pore blocker. Thus, Li et al. reported the one-pot construction of functional MSNs for the tumor acid-triggered synergistic chemotherapy of glioblastoma [59]. To this aim DOX was conjugated to MSNs through acid-cleavable hydrazone bonds and camptothecin (CPT) was loaded into the pores of MSNs. In the release studies there can be seen that at pH 6.5, similar to that in tumor tissues, and at pH 5.0 (similar to that of endo/lysosomes of cancer cells) a fast DOX release took place obeying the hydrolysis of hydrazone bonds kinetics. This allowed the release of CPT. The simultaneous delivery into tumor cells of CPT plus DOX provided good expectations in the treatment of glioblastoma (Entry 1, Table 3). In another work, Liu et al. reported the use different combinations of well-known chemotherapeutics and profited of their different solubility and adhesion to develop a strategy for the sequential loading in which the latter acts also as capping moiety [60]. The strong electrostatic interaction exhibited by DOX-SiO$_2$ pair was used to maintain the other hydrophobic drug within the pores. It is also noteworthy that DOX release is slower in the case of the MSNs containing both drugs, which is justified by unfavorable kinetics for alternating outflow. The therapeutic profile of the double loaded MSNs was studied against alveolar cancerous cells showing an enhanced apoptotic effect than the obtained for the single drug model (Entry 2, Table 3).

Although the controlled multiple loading-release of two or more chemical entities within the channels of the MSNs is not simple, the ease of post-loading functionalization turn them into a valuable rigid and robust candidate for the construction of containment coatings able to avoid drug leakage. On the first reported MSNs-based codeelivery a model fluorophore was placed in the pores while a pH disintegrable cisplatin (DDP) containing a polyelectrolyte multilayer was used as coating shell with both load and protective roles [61]. In this work Wan et al. employed alternating negative–positive polymers to coat the cationic amino functionalized MSNs, but adding Pt
complexes in between layers to effectively trap them into the polymeric matrix. The polymeric shell so designed proved to be broken in acidic media because of the disappearance of the negative carboxylate groups. This produced the electrostatic interaction disappearance and the release amino containing CDDP complex (Entry 3, Table 3). A similar strategy was employed by Li et al. for the encapsulation and delivery of CPT and DOX in HMSNs [62]. Their design was based on electrostatic adhesion of ZnO quantum dots (QD) to the carboxylate-functionalized surface of the silica nanoparticles. In this work the authors also performed sequential loading of cytotoxic drugs in which DOX was the latter incorporated compound. In vitro cellular assays with the material against A549 and MCF-7 cellular lines showed interesting enhanced apoptotic effect when using the QD-CPT-DOX system (Entry 4, Table 3). It is also remarkable that the QD-DOX combination performs significantly better than free DOX; this could be due to a delivery effect alone or to a combined effect of DOX with the possible toxicity of the QD employed.

Another known approach for a controlled release is based on the MSNs coating with a lipid layer. These hybrid materials combine the loading capacity and robustness of silica with the outstanding protective effect of micelles. Along this line, Nel et al. employed lipid coated MSN loaded with Paclitaxel (PTX) and Gemcitabine (GEM), two first line chemicals for pancreatic cancer treatment, for its evaluation in mice [63]. The in vivo results with this material showed an increased therapeutic effect compared with the separate drugs, including commercial nanomedicines. In vivo assays indicated a slight tumor volume regression when treated with their double drug system oppositely to the tumor stasis obtained with the rest of single-drug chemotherapeutics (Entry 5, Table 3). As there will be reviewed below, the use of additional sensitizing agents in combination with anti-proliferative drugs could also exert reversal drug resistance. The incorporation of various hydrophobic compounds into the pores of MSNs could be also successfully achieved by performing the synthesis of MSNs with surfactant-stabilized chemotherapeutics. In most of reported literature on this topic, the therapeutic effect usually relies in a single drug. However the presence of a surfactant has demonstrated to act as cellular sensitizer through destabilization of cellular membranes or by being the substrate for drug efflux pumps. In a pioneering work, Vivero-Escoto's group used as prepared MSNs containing a nontoxic phosphonate surfactant, which were loaded with resveratrol (RVT) as hydrophobic model molecule [64]. The loading was achieved by soaking the surfactant containing MSNs into a concentrated solution of RVT, which allowed an effective loading within the surfactant. The in vitro evaluation against HeLa cell line showed a pH dependent release of RVT together with a slight decrease on the cellular viability (Entry 6, Table 3). Almost simultaneously, He et al. reported the use of an aqueous DOX stabilized with cetyltrimethylammonium bromide (CTAB) micelles for its use as mesopore template. The resulting DOX-CTAB loaded MSNs were tested in vitro against MCF-7 resistant and nonresistant cell lines [65]. In this case CTAB was chosen as surfactant as it provided the highest lethal effect on cells [69]. The combination of both drug and sensitizer

<table>
<thead>
<tr>
<th>Entry</th>
<th>Delivery system</th>
<th>Drugs(s) Loaded</th>
<th>Drug location</th>
<th>Release system</th>
<th>Release stimuli</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MSNs</td>
<td>DOX grafted via hydrazone bond CPT</td>
<td>Pore and shell</td>
<td>pH-dependent hydrazone cleavage</td>
<td>pH decrease</td>
<td>[59]</td>
</tr>
<tr>
<td>2</td>
<td>MSNs</td>
<td>RPM + DOX PTX + DOX</td>
<td>Pore Pore Pore DOX capping</td>
<td>Pore DOX capping</td>
<td>pH decrease</td>
<td>[60]</td>
</tr>
<tr>
<td>3</td>
<td>MSNs</td>
<td>CDDP RHD B</td>
<td>Shell Pore</td>
<td>Polyelectrolyte pH sensitive shell</td>
<td>pH decrease</td>
<td>[61]</td>
</tr>
<tr>
<td>4</td>
<td>Hollow MSN</td>
<td>CPT DOX</td>
<td>Pore</td>
<td>Electrostatic assembly with quantum dots</td>
<td>pH decrease</td>
<td>[62]</td>
</tr>
<tr>
<td>5</td>
<td>MSNs</td>
<td>PTX GEM</td>
<td>Shell Pore</td>
<td>Lipid shell</td>
<td>None</td>
<td>[63]</td>
</tr>
<tr>
<td>6</td>
<td>MSNs</td>
<td>Surfactant (PO₄³⁻ based) RVT</td>
<td>Pore None</td>
<td>pH dependent surfactant release</td>
<td>[64]</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>MSNs</td>
<td>Surfactant (CTAB) DOX</td>
<td>Pore Surfactant None</td>
<td>pH dependent surfactant release</td>
<td>[65]</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>MSNs</td>
<td>Surfactant-lipid hybrid IRN</td>
<td>Shell Pore</td>
<td>Lipid shell</td>
<td>pH dependent shell cleavage</td>
<td>[66]</td>
</tr>
<tr>
<td>9</td>
<td>MSNs</td>
<td>TCP-PEG-succinate DOX</td>
<td>Shell Pore</td>
<td>Redox (shell) Lipid shell linked by disulfide bond</td>
<td>pH decrease</td>
<td>[67]</td>
</tr>
<tr>
<td>10</td>
<td>MSNs</td>
<td>DOX CA4</td>
<td>Pore None</td>
<td>pH decrease</td>
<td>None</td>
<td>[68]</td>
</tr>
</tbody>
</table>

provided better results in combination than independently, as demonstrated by cell viability studies (Entry 7, Table 3).

The satisfactory results derived from the combination of cytotoxic plus drug efflux pump substrates encouraged other research groups to develop different systems to entrust both effects using lower-cost components than siRNAs. The reported example by Zhang et al. employed irinotecan (IRN) loaded MSNs coated with a Pluronic® (Basf Corporation, Ludwigshafen, Germany) containing phospholipid shell [66]. The P123 block copolymer conjugated to the hybrid phospholipid was able to disrupt the drug efflux pump resistant route. Furthermore the lipid shell employed provided some additional features such as higher dispersability due to Pluronic® hydrophilic component and the controlled release behavior due to the pH-sensitive coating by the lipid layer; the pH-sensitivity and the effect of membrane-destabilizing agents were elegantly demonstrated in drug-release studies, which showed increased uptake for the lipid formulation with an expected increase in the amount of internalized IRN and reduction of viability on MCF-7 cellular line (Entry 8, Table 3).

A similar research work by the group of Wang reported a similar device with DOX loaded MSN coated with a lipid shell [67]. The authors included a tocopherol-PEG hybrid inlaid within the lipid coating as substrate for the Pgp protein and a redox sensitive shell on reducible disulfide bridges between the MSN and the lipid molecules. The dual pH-redox combined release systems provided a stable and effective pore capping as seen on the nitrogen adsorption isotherms and measured pore diameters. The in vitro study with MCF-7 cell line showed an enhanced uptake and cellular death assumed by reversal drug resistance (Entry 9, Table 3). Along this line, Li et al. in a recent work demonstrated that use of other drug combinations, such as DOX plus antiangiogenic combretastatin A4 (CA4), loaded into MSNs also provide an enhanced apoptotic effect and delay on tumor growth [68]. The system was further targeted with the RGD peptide as guidance moiety. The apoptotic effect in vitro showed similar values for both DOX and DOX+CA4 loaded nanoparticles, consequence of the low inherent toxicity of CA4 however in vivo data showed the clear inhibition of tumor growth, demonstrating the potential of this strategy (Entry 10, Table 3).

4. Combination with apoptosis induced by physical methods

Another developing approach for the dual treatment of cancer is the combination of classic cytotoxic drugs with an additional effect, generated by exposure to a physical stimulus; either able to sensitize the cancerous tissue to the effect of chemotherapeutic or to induce an additional and independent apoptosis route. As previously discussed, combinations of cellular killing pathways provokes enhanced therapeutic profiles in drug resistant cancerous cells. Furthermore, the application of the apoptosis induced by physical methods to MSNs based nanocarriers is highly interesting as the triggering of this apoptotic pathway would only occur in the areas where the nanocarrier is, thus minimizing undesired side effects.

4.1. Photodynamic therapy

Photodynamic therapy (PDT) is based on the use of a substance, a photosensitizer (PS), able to absorb concrete wavelengths of electromagnetic radiation. The generated excited state is able to transfer this energy to molecules containing oxygen atoms and produce the so-called reactive oxygen species (ROS). These high-energy compounds are potential cytotoxics as their high reactivity and low selectivity generate irreversible cellular damage, thus favoring cellular death (Figure 2(a)) [70]. The use of PDT is improved for biomedical applications when two-photon absorption in the visible (Vis) or near infrared (NIR) regions is employed, since a deeper tissue penetration together with a reduction of the risks associated with ultraviolet (UV) light handling needed for single photon PDT is obtained [71].

Although PDT based on mesoporous silica platforms is a quite exploited research field, the authors’ interests have been mainly focused in the development of new hybrids with different nature PS rather than in their combination with anti-proliferative drugs. For instance, Gary-Bobo et al. used mannose targeted MSNs functionalized with a water-soluble porphyrin sulfonate PS and loaded with CPT [72]. As expected, the CPT exerted a cancerostatic action reducing the viability of
all cellular lines tested to ca. 60%. Nonetheless, the combined effect with PDT dropped the survival of HCT116 and MDA-MB-231 to ca. 20% while Capan-1 line showed complete cellular death, thus probing a synergistic effect (Entry 1, Table 4).

In a similar approach reported by Yang et al. a porphyrin based PS (Chlorin e6) was introduced within the silica matrix by reaction with an alkoxysilane followed by cocondensation with the silica precursor. Then, the so obtained nanorods were loaded with DOX and evaluated against several cell lines [73]. The reported results showed a synergistic effect higher than the theoretical additive effect of both monotherapies, demonstrating again the potential of combined therapy (Entry 2, Table 4).

A nice implementation of the system, done by Chen et al., employed Zn-porphyrin PS as a pore blocker [74]. In their design, MSNs loaded with DOX were functionalized with double pH features able to respond to both extra- and intracellular acidic microenvironments. The pore capping unit, formed by the PS, the pH sensitive cis-aconitic moiety and polyethylene-glycol (PEG), was electrostatically linked to histidine decorated MSN which gave an acidic cleavable bonding between both subunits. Unfortunately, no relevant studies regarding the therapeutic effect are provided in this work (Entry 3, Table 4). A recent work by Vivero-Escoto and El-Nagheeb also demonstrated the potency of the combined chemophotodynamic therapy by reducing the survival of HeLa cells. For so, they employed MSNs loaded with a combination of a phthalocyanine as sensitizer and CDDP as chemotherapeutic [75]. In this work, the authors provided clear data proving that combination therapy is much more effective than separated or simultaneously applied monotherapies (Entry 4, Table 4).

Apart from the organic-based sensitizers, there is also an interesting approach based on radiation upconversion luminescence implemented for drug delivery reported by Liu et al. In this strategy they use lanthanide-doped particles for transformation of the incident NIR light into high-energy UV photons. The group reported two different non-MSNs based models in which the UV emission performs the release of a Pt prodruk linked through an UV sensitive bond [87] or generation of ROS upon excitation of TiO2 particles conveniently placed on the surface of their upconversion nanodevices [88]. Furthermore, the authors have employed the exceptional properties of light upconversion to design a system in which the photoactive species (NaYF4:Yb3+/Er3+) are embedded in a mesoporous silica further coated with a pNIPAM copolymer layer with pH- and thermoresponsive properties. In this last example they effectively confined DOX within the pores in which premature cargo release was avoided by the outer hydrogel layer [76]. Although the object of this article was more focused on the luminescent properties rather than the therapeutic aspect of combination of PDT with chemotherapy, the development of those kinds of platforms could enable multimodal therapeutics plus interesting bioimaging properties (Entry 5, Table 4).

### 4.2. Photothermal therapy

Unlike the PDT in which the energy from light radiation should be energetic enough to convert low reactive triplet oxygen molecule into the high reactive singlet oxygen one, photothermal therapy (PTT) is based in a different phenomenon. In
this case the infrared radiation, highly related with the vibrational excitation of molecules, acts over the PS and, through thermal relaxation, induces local heating. As it is known, any increase of local temperature within the cell triggers either an apoptotic mechanism or, if more intense, the thermal degradation of the tissue. Thermal induced apoptosis is usually balanced by expression of heat-shock proteins and, as expected, the combination of both thermal plus chemical apoptotic pathways could be harnessed to increase the efficiency compared to analogous monotherapy treatments (Figure 2(b)).

Along this section different materials showing photothermal effect will be reviewed, but in general PTT is usually based on plasmonic resonance for inorganic materials and extended conjugation for organic ones. The most recurrent material for photothermal treatments are gold nanoparticles (AuNPs), whose ease of functionalization and known plasmonic resonance turn them into a wide spread material. In the pioneer work by Chen et al. they employed Au nanorods (AuNRs) as core coated by a MSN shell for the loading and delivery of DOX [77]. Their studies showed a typical pH dependent DOX release from the shell mesopores which was substantially increased when NIR lighting was applied. In vitro studies with A549 cell line showed an enhanced cellular death when both chemo- and thermal effects were combined. Furthermore, authors claimed that the punctual photothermal effect assisted the endosomal escape, thus allowing the loaded drug to better diffuse throughout the cell (Entry 6, Table 4). In another work, Shi’s group used anionic AuNRs electrostatically bound to a cationic Fe₃O₄@MSN nanoparticle for a similar purpose [78]. Although there is no data about combined therapy in vivo, the authors provided a nice chart relationship between induced heating and chemotherapy. They showed that below 39ºC and above 45ºC the main operating effects were cytotoxicity and hyperthermia respectively, while at 42ºC the combined effect was increased respect to the different independent therapies. However, the local release of DOX may still perform therapeutic action over the remaining cancerous mass, thus increasing the potentially of this therapy (Entry 7, Table 4).

Although Au is one of the most readily available materials for the construction of hybrids with mesoporous silica matrices, there are other many chemical sensitizers different in nature able to perform this task. In one example reported by Huang et al., a graphene sheet behaving as PS was coated with mesoporous silica and loaded with DOX (Entry 8, Table 4) [79]. Another organic species reported to behave as PS is polypyrrole (PPy), as it bears an extended conjugation similar to that of graphene, although linear in this case. Zhang et al. employed polypyrrole-polyacrylic acid (PPy-PAA) nanoparticles as growing seeds for MSNs construction, which were successfully loaded with DOX as cytotoxic [80]. The authors reported an enhanced apoptosis when compared with raw cytotoxic similar to those obtained with other reported systems but with a clear advantage, the lower cost of PS in comparison to other reported systems (Entry 9, Table 4). In addition to the graphene and PPy sensitizers, the organic sensitizer cypate (CYP) has also been satisfactorily employed for the construction of DOX containing micelles with photothermal properties [89]. Besides Au, other inorganic species could also act as PS, such as Cu₅ [90]. Thus, Lu et al. have efficiently employed core@shell Cu₅@MSN nanoparticles for the delivery of DOX for combined treatment of HeLa cell line with efficiencies comparable to those obtained with neat chemotherapeutic agent (Entry 10, Table 4) [81].

Another relevant contribution to the state of the art in multitherapeutic use of MSNs was reported by Zhang et al. In their model they used Cu₅ embedded in a mesoporous silica matrix able to respond to NIR irradiation to achieve thermal excitation at 980 nm. The authors employed a mixture of curcumin (CUR) and DOX which have demonstrated to perform a toxic synergistic action [91]. The system was designed by loading of CUR within the pores and functionalizing the outermost surface by anchoring a single strand DNA. This DNA was hybridized with the recognition aptamer AS1411, which was able to perform cellular recognition and internalization on MCF-7 breast line. Additionally, the cytotoxic drug DOX was intercalated within the double strand DNA to complete the system and allow double thermal release of CUR and DOX. (Entry 11, Table 4) [82].

Despite the existence of organic sensitizers, Au is by far the most reported one. A number of examples also describe systems able to perform additional diagnostic features that could be also employed for combined therapy. As an example, the design by Lv et al. includes an inner lanthanide oxide particle for providing luminescent properties to the system. In this case the photothermal property resides in Au₂₅₃ clusters embedded together with DOX within the pores. The system is completed with a thermal sensitive poly(N-isopropyl acrylamide)metacrylic acid (poly(NIPAm-MAA)) (Entry 12, Table 4) [83]. In another example, Zhang et al. [84] reported the use a pH sensitive imine bond to link DOX to the surface of AuNR@MSN which may be photothermally accelerated. Again, the obtained results are in concordance with the previous observed behavior of cumulative apoptotic effects (Entry 13, Table 4).

Two very recent examples including in vivo experiments support the hypothesis of combined light-induced photochemotherapy as a potential improvement for future therapeutics. In one example by Zhang et al. an octopus-like, Janus type Au@MSN nanoparticle was used to achieve complete tumor remission of liver tumors on mice when applying both therapeutic effects together (Entry 14, Table 4) [85]. The second recent example, by Wang et al., employed the synergistic therapy for melanoma treatment using several sizes of rod-type Au@MSN@Au nanohybrids loaded with docetaxel (DTX) (Entry 15, Table 4). Their studies showed too complete remission of tumor when using combination therapy. Although this is out of the scope of the current review, which is mainly focused on MSNs as delivery agents, the authors would like to let the readers know that there is an interesting review about effects of synergistic combination of gene and PTT [92].

### 4.3. Magnetic hyperthermia

Another well-known effect for enabling the thermal-mediated apoptosis of tumor cells is magnetic induced hyperthermia, which is efficiently generated by stimulation of the sensitive
material to alternating magnetic fields (AMF). Although the use of magnetic materials has become widely employed and extensively reviewed, there are scarcely studies regarding combined thermo and chemotherapy with mesoporous magnetic materials. Lu et al. reported the synthesis of HMSNs encapsulating iron oxide nanoparticles (IONPs) [93], which allowed the resulting nanocapsule acting as DOX delivery system. In fact, the hollow interiors of HMSNs permitted hosting high chemotherapeutic amounts; for further details into the comparison of HSMSNs and MSNs please check references included in the review authored by Tang et al. [12] Upon exposure to an AMF, IONPs promoted DOX release and also elevated the temperature of the surrounding media to clinical hyperthermia levels (41–46°C). Tao and Zhu prepared magnetic MSNs (mMSNs) by encapsulating Fe$_3$O$_4$ nanoparticles in MSNs and DOX was used as anticancer drug to evaluate the drug delivery capability of those mMSNs [94]. DOX-loaded in mMSNs was released in the medium at pH 5.0, similar to that in the intracellular endo/lysosomes. In addition, mMSNs efficiently generated heat upon exposure to an AMF due to their superparamagnetic performance. The two systems described herein provide promising nanoplatforms for the combination of chemotherapy and hyperthermia for antitumor therapy. In case the reader wished to deep into this topic there are several interesting reviews available for further reading [95–98].

4.4. Radiotherapy

Besides chemotherapy and surgical removal of malignant mass, radiotherapy is also an important pillar of cancer treatment. It is based on the application of highly ionizing and penetrating radiation able to destroy the tumor tissue; although, again, there is a lack of selectivity between health and malignant tissues. Fortunately, the therapeutic radiation could be focused to reduce its effect on neighbor tissues, but the selective effect on cancer cells is still a chimera. Nonetheless, the development of nanotechnology could advance the future radiotherapies by basing them in the preferential accumulation within the solid tumor masses possessing EPR effect.

Most reported radioactive nanodevices are designed for diagnosis purposes [99] and usually the amount of radioactive material is not enough to achieve a therapeutic action. However the advance in synthetic procedures may provide in the near future nice platforms for nanotransported sources of radioactivity (⁶⁰Co, ¹³¹I, etc.) [100,101] even on mesoporous materials [102] thus enabling the on-site application of radio and chemotherapy or even on-demand combinations.

Shen et al. reported a MSN-based nanosystem for reversing MDR by synergetic chemoradiotherapy [103]. For this purpose MSNs were loaded with topotecan (TPT), a typical radiosensitizing drug, and the outermost surface of the nanoparticles was decorated with PEG to improve biocompatibility. MSNs performed the transport of TPT into MDR cells while passing the Pgp pumps, and chemodrug-sensitized radiation improvement was directly accomplished within the cells by high energy X-ray irradiation. This was in vitro demonstrated using MCF-7/ADR cells (adriamycin-resistant breast cancer cells), which experienced and increase in necrosis/apoptosis enhanced by DNA damage. The same research group reported the synthesis of rattle structure upconversion core/mesoporous silica nanoteranostics functionalized with TAT ligand to efficiently target cell nucleus [104]. The radiosensitizing drug mitomycin C (MMC) was confined into the nanosystems to be delivered into the nucleus upon exposure to high energy X-ray irradiation. In vitro and in vivo assays demonstrated the enhanced treatment efficacy by the intranuclear radiosensitization than the extracellular and intracellular ones in killing cancer cells and inhibiting tumor growth.

Very recently, Ma et al. reported a novel B$_2$S$_3$-based nanoparticle, which is a well-known candidate as radiosensitizer upon exposure to X-ray irradiation, coated with a mesoporous silica shell and loaded with DOX into the mesopores [105]. In vitro assays demonstrated that the nanosystems exhibited on demand pH DOX responsive release and improved the therapeutic effect against MDR cancer cells. Besides, in vitro and in vivo experiments evidenced that the nanoplatofms could notably increase the interstitial $^{32}$P radionuclide radiotherapy in the solid tumor. These findings revealed this novel nanosystem as a promising alternative for the synergistic combination of chemointerstitial radiotherapy.

5. Conclusion

Many of the recent biomedical research efforts are being dedicated to develop novel strategies able to overcome the current limitations of cancer therapy. Nanocarriers, namely MSNs, are outstanding and versatile candidates to achieve this goal. Advanced strategies have been designed to selectively targeting and killing cancer cells that have acquired resistance to usual chemotherapeutics. All the strategies reviewed in this manuscript clearly evidence that the main goal is to develop a library of nanotherapeutics that allow to increase cancer cell death while reducing the overall dosage needed for a successful treatment of difficult-to-treat tumors. Up to date only in vitro and preliminary in vivo assays have been performed, but much scientific effort must be still done before entering clinical trials. Some of the issues that must be addressed are determination of optimal size and shape for therapeutic application and clinical testing, establishment of dosage scales for murine and human experiments in order to minimize acute toxicity, determination of long-term toxicity and genotoxicity, studies about distribution in tissues and organs and an extensive study on MSNs’ metabolism and excretion. Additionally, precise preclinical host-guest loading-release studies must be accomplished to set standards in drug delivery and controlled release. But one of the most important issues may arise from the exceptional modularity of MSNs, because most of designs include fragments not fully evaluated components (the MSNs themselves are not fully clinically evaluated); such as set of linkers, building blocks and even other particles that may give acute or chronic toxicities if systemic administration is systematically employed [106–112].

6. Expert opinion

Although MSNs have been widely exploited for the design of sophisticated nanodevices for antitumor therapy, they still
remain in the forefront of scientific research, as MSNs allow unique modifications to include new features which otherwise could not be incorporated to classic treatments. One of the most recent strategies, focused on improving targeting, is the enhancement of cellular/tissue recognition through ligands selection to specifically match the receptors present in diseased cells.

In the clinical practice, the combination of specific targeting and potent biological effects given by biomacromolecules (antibodies, aptamers, proteins, new generation peptides, etc.) with antitumor drugs has opened the way to more efficient cancer treatments; thus improving the effect of classic chemotherapeutics, mainly for the treatment of relapsing and MDR tumors [113]. Nanocarriers, extraordinary platforms to combine in a single entity more than one therapeutic effect, are of special interest because they could be designed to deliver and then exert those effects simultaneously. Although this research field is still at its infancy and thus many efforts should still be made to achieve a real clinical application, some basic concepts for nanocarrier development are clear.

The first examples of matching recognition were achieved via multi-ligand recognition onto diseased cells. Within this context, although contributions based on MSNs are not prolific, there are highly interesting approaches reported using different nanosystems. For example, RGD-based peptide combinations [26,114,115] (Entries 1 and 2, Table 5) or dual-targeted formulations employing antibodies have been developed (Entries 8–10, Table 5) [116–118]. Albeit some antibody-based dual-targeting nanodevices involving angiogenesis (vascular-to-cellular) or membrane-to-nucleus are interesting, there are relevant issues that remain unsolved: the unknown immunogenic effect and an undemonstrated specificity toward the desired target cell. Another relevant approach, based on dual-aptamer recognition, has also been described for the specific recognition of different HER2 and MUC1 positive breast adenocarcinomas (Entry 3, Table 5) [119]. Perhaps the combination of low immunogenicity and high specificity of aptamers could give access to a new family of therapeutic nanocarriers with promising specificity toward particular tumor cell lines. Besides, aptamers offer several other advantages such as higher robustness than antibodies, which difficult manipulation and low stability could lead to false findings with devastating effects [120]. In any case, the possible combinations arisen from the use of several biomacromolecules as targeting moieties are practically unlimited. Then, for a reasonable development of targeted devices a concrete set of different specific-to-cell fragments would be highly appreciated by researchers in order to maximize the recognition process.

Another crucial aspect of cancer therapy is the evolution of therapeutics against multidrug resistant cells. For so, the strategies regarding codelivery and combination therapies show promising results, although still not fully developed. This issue remains unsolved in the clinical field but could be addressed by the delivery of two (or more) potent antiproliferative compounds; mainly when the combination simultaneously disrupts different replication routes. An overview of some clinical trials indicates that the combination of

Table 5. Non MSNs-based nanosystems designed up to date for combined therapy.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Delivery system</th>
<th>Primary targeting</th>
<th>Secondary targeting</th>
<th>Therapeutic drug loaded</th>
<th>Cellular line</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Liposome</td>
<td>c(RGDfC) peptide</td>
<td>CDAEWDV5 peptide</td>
<td>None</td>
<td>4T1, MDA-MB-231 (breast)</td>
<td>[114]</td>
</tr>
<tr>
<td>2</td>
<td>Polymeric</td>
<td>RGD peptide</td>
<td>Interleukin 13 peptide</td>
<td>Cou-6</td>
<td>C6 HUVEC</td>
<td>[115]</td>
</tr>
<tr>
<td>3</td>
<td>Silica + Magnetic beads</td>
<td>HER2 aptamer</td>
<td>MUC1 aptamer</td>
<td>None</td>
<td>MCF-7, T47D, BT-474 and SK-BR-3 (breast)</td>
<td>[119]</td>
</tr>
</tbody>
</table>

Interfering RNAs and cytotoxic codelivery

<table>
<thead>
<tr>
<th>Entry</th>
<th>Delivery system</th>
<th>siRNA</th>
<th>Tumor targeting</th>
<th>Therapeutic drug loaded</th>
<th>Cellular line</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Polymeric</td>
<td>Bcl-2</td>
<td>Folic acid</td>
<td>DOX</td>
<td>MCF-7 (breast)</td>
<td>[121]</td>
</tr>
<tr>
<td>5</td>
<td>Polymeric</td>
<td>Bcl-2</td>
<td>Folic acid</td>
<td>DOX</td>
<td>MCF-7 (breast)</td>
<td>[122]</td>
</tr>
<tr>
<td>6</td>
<td>Polymeric</td>
<td>Hsp90</td>
<td>LPLTPLP peptide</td>
<td>DTX</td>
<td>A549 (lung)</td>
<td>[123]</td>
</tr>
<tr>
<td>7</td>
<td>Polymeric</td>
<td>Survivin</td>
<td>Apoptosis blocker</td>
<td>None</td>
<td>DOX</td>
<td>[124]</td>
</tr>
</tbody>
</table>

Monoclonal antibodies and cytotoxic codelivery

<table>
<thead>
<tr>
<th>Entry</th>
<th>Delivery system</th>
<th>Monoclonal antibody</th>
<th>Tumor targeting</th>
<th>Therapeutic drug loaded</th>
<th>Cellular line</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Liposome</td>
<td>Anti GD2 (sialoganglioside)</td>
<td>NGR peptide (CD13, tumor vessel)</td>
<td>DOX</td>
<td>HTLA, SH-SY5Y and NXS2 (neuroblastoma)</td>
<td>[116]</td>
</tr>
<tr>
<td>9</td>
<td>Polymeric</td>
<td>Trastuzumab</td>
<td>Folic acid</td>
<td>DOX</td>
<td>MCF-7 and BT-474 (breast)</td>
<td>[117]</td>
</tr>
<tr>
<td>10</td>
<td>Polymeric</td>
<td>Gemcitabine</td>
<td>None</td>
<td>OXA</td>
<td>AsPC1, BxPc3 (pancreas)</td>
<td>[118]</td>
</tr>
</tbody>
</table>

Drug efflux pump substrate plus therapeutic drug codelivery

<table>
<thead>
<tr>
<th>Entry</th>
<th>Delivery system</th>
<th>Drug efflux substrate</th>
<th>Drug role</th>
<th>Therapeutic drug loaded</th>
<th>Cellular line</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>Polymeric</td>
<td>Pgp modulator</td>
<td>LMT</td>
<td>Brain sections of SE48H rats</td>
<td>[125]</td>
<td></td>
</tr>
</tbody>
</table>

COU-6: coumarin 6; DOX: doxorubicin; DTX: docetaxel; PTX: paclitaxel; OXA: oxaliplatin; LMT: lamotrigine (antiepileptic drug).
therapeutic compounds of different nature improves patient’s life expectancy, although sometimes it is hampered by the intensity of side effects. Fortunately, the development of codelivery strategies onto targeted-containing nanocarriers could mitigate the therapy aggressiveness. However, more emphasis should be done on the study about the optimal drug ratio and drug combinations as employed in the clinical practice. This topic, which was smartly introduced by Johnson et al., may establish relevant future guidelines for the development of more efficient nanomedicines [126]. To successfully adapt all these strategies to MSNs, they must be combined with different stimuli-responsive mechanisms, which fortunately are well developed [127].

An alternative and promising strategy is the simultaneous disruption of the replication route together with a crucial antiproliferative pathway. Apart from the systems already reviewed along the manuscript, nanoplatforms are able to deliver interfering-based therapeutics together with cytotoxics (Entries 4 and 5, Table 5) [121,122] have demonstrated that gene down-regulation plus an apoptotic mechanism have potent antiproliferative effects. Furthermore, this approach enables an extraordinary potential therapeutic effect if multiple gene silencing are considered. So, the use of several nucleic acids able to disrupt several critical cellular pathways simultaneously [128] could boost the fight against cancer. Following this idea, the combination therapy of cytotoxic with down-expression of antiproliferotic related proteins could be an attractive approach. In the literature it has been reported that a single LPLTPLP peptide is able to play targeting and inhibition over heat-shock protein Hsp90 (Entry 6, Table 5) [53,123]. Also, the delivery of survivin siRNA shows nice results in combination with chemotherapeutic agents (Entry 7, Table 5) [124]. Nevertheless, all reported cases aligned onto this strategy have only preclinical results and then knowledge on potential side effects are required before new combinations are developed.

It has been also reviewed that the combination of a single antiproliferative compound with a sensitizer provides a substantial increase of the apoptotic effect of the chosen drug. This strategy, although less efficient than the codelivery of cytotoxics, could be interesting when the toxicity reached by the drug combination is not suitable for its use in systemic therapies. Furthermore, the presence of surfactants provides an additional advantage as the use of pore caps or coatings is usually not required. Following this idea Liu et al. have also reported that disruption of Pgp increases the therapeutic effect of antiproliferative drugs (Entry 11, Table 5) [125].

As a conclusion of the reviewed codelivery approaches, it is possible to assume that most of the different nanosystems have increased the therapeutic profile with respect to the chosen cytotoxic drugs; which are now able to defeat and eradicate efficiently most of cancerous cell lines when tested in vitro. Unfortunately, although the results shown are promising, the reality is that most of these research do not have continuity in clinical studies; because of this, we strongly believe that future efforts should be addressed toward the evaluation of the most efficient combinations of basic systems more than to the development of more complex ones, with little or no preference for biogenic components which are always of preference.

The combination of chemotherapy with apoptosis induced by physical methods is also an emergent discipline in the development of nanomedicines for future cancer therapies. The main advantages of this strategy are both low toxicity and ease to control intensity and location of stimuli, although the range of action might be restricted to superficial or easily reachable tissues. Despite the activation is innocuous for both PDT and PTT, there are several problems associated to the application of these combined therapies. These include the potential risk of long-term sensitivity of patients to remaining photoactive compounds within the organism, the development of resistance to PDT by the increment of antioxidant compounds, or long-term toxicities to either PS or to the different employed components [71,92]. Regarding photo-thermal ablation there is also potential risk of inducing damages in neighbor healthy tissues. Nevertheless, the promising results obtained with light-responsive materials in combination with chemotherapy make relevant the development of new formulations for the implementation of current therapies, highlighting those in combination with those previously reviewed: targeting, gene knock-down and/or codelivery.

The development of nanotechnology may also provide many different systems with either enhanced selectivity or dual action modes. For example the combination of known ultrasound-responsive MSNs based drug delivery [129] with sonosensitive compounds [130], or radiochemotherapy would open up promising expectations in the development of new therapeutic possibilities for treatment of deep tumors in an easy fashion.

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**Declaration of interest**

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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