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# Drug delivery to resistant tumors: the potential of poly(alkyl cyanoacrylate) nanoparticles☆

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**Abstract**

Simultaneous cellular resistance to multiple lipophilic drugs represents a major problem in cancer chemotherapy. This drug resistance may appear clinically either as a lack of tumor size reduction or as the occurrence of clinical relapse after an initial positive response to antitumor treatment. The resistance mechanism can have different origins either directly linked to specific mechanisms developed by the tumor tissue or connected to the more general problem of distribution of a drug towards its targeted tissue. The purpose of this paper is to summarize the results of the use of poly(alkyl cyanoacrylate) nanoparticles to overcome multidrug resistance (MDR) phenomena at both the cellular and the non-cellular level.


*Keywords:* Nanoparticles; Poly(alkylcyanoacrylate); Anticancer drug; Resistant cancer

## 1. Introduction

In chemotherapy, pharmacologically active concentrations of an anticancer drug in the tumor tissue are often reached at the expense of massive contamination of the rest of the body. This poor specificity creates a toxicological problem that represents a serious obstacle to effective antitumor therapy. In addition, the occurrence of resistance phenomena increases the problem of tumor treatments. Thus, in clinics, the occurrence of multidrug resistance (MDR) may appear either as a lack of tumor size reduction or as a clinical relapse after an initial positive response to antitumor treatment [1]. As illustrated in Fig. 1, the resistance mechanism can have different origins. In the tumor tissue, it can be either directly linked to specific mechanisms developed by the tumor cells or it can be connected to the physiology of the tumor tissue, including a poor vasculature and unsuitable physicochemical conditions [2–5]. Outside the tumor tissue, the resistance to chemotherapy can be due to the more general problem of the distribution of a drug relative to its targeted tissue [6]. To overcome drug resistance many attempts have been made using strategies that consider the more general problem of the control of the drug biodistribution either at the cellular level or at the tissue level [2,7]. The purpose of this paper is to summarize the results of the use of nanoparticles to overcome MDR phenomena occurring at both the cellular and the non-cellular level. Thus, the first part will give an overview of the main results obtained with nanoparticles designed to over-

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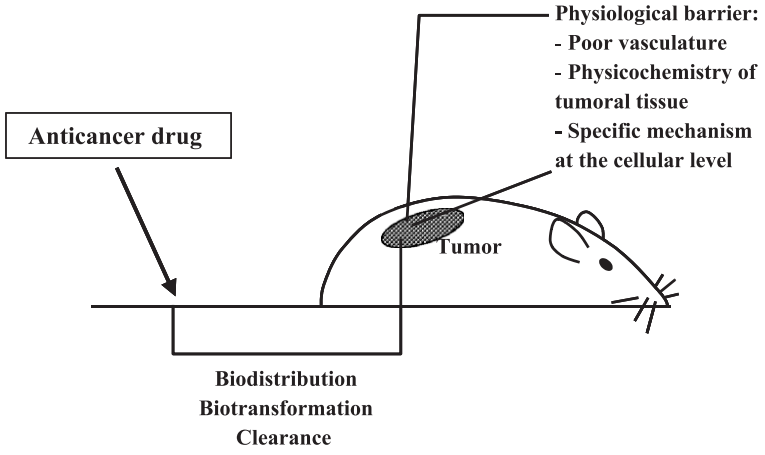


Fig. 1. Main causes of clinical observations of multidrug resistance during anticancer therapy.

come specific resistance at a cellular level. In the second part, the different nanoparticles designed to achieve a better control of the biodistribution of drugs towards tumoral tissue will be described.

## 2. The potential of nanoparticles to overcome multidrug resistance at the cellular level

Tumor cells can specifically develop simultaneous resistance to multiple lipophilic compounds [1,2]. For instance, cellular resistance to anthracyclines has been attributed to an active drug efflux from resistant cells, linked to the presence of transmembrane P-glycoprotein (P-gp), which was not detectable in the parent drug-sensitive cell line [8]. As illustrated in Fig. 2, drugs such as doxorubicin appear to enter the cell by passive diffusion through the lipid bilayer. With resistant cells, upon entering the cell, the drug binds to P-gp and is pumped out of the cell [8,9]. To circumvent this MDR at the cellular level, many authors have proposed the use of competitive inhib-

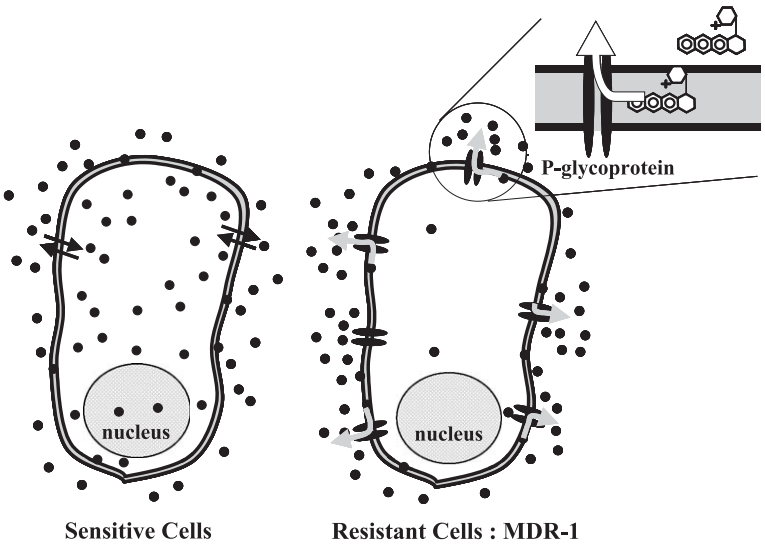


Fig. 2. Schematic representations of the penetration of an anticancer drug in sensitive and MDR-1-resistant cells.

itors such as the calcium channel blocker, verapamil [2]. However, the clinical use of verapamil to overcome MDR is limited due to the serious adverse effects of this compound. Another strategy suggested for delivery of anticancer drugs, with the aim of overcoming resistance phenomena, is association of the drug with colloidal carriers such as nanoparticles [10,11]. The rationale behind this strategy is to increase the intracellular concentration of the drug using endocytosis.

Doxorubicin, an anticancer drug widely used in cancer therapy and a known substrate of P-gp, was encapsulated in various types of nanoparticles [12–14]. The sensitivity of resistant cells to the doxorubicin-loaded nanoparticles was then evaluated by measuring the cytotoxic effect produced by increasing the concentration of the doxorubicin-loaded nanoparticles. Resistant cells treated with alginate or poly (lactide-co-glycolide) nanoparticles showed the same sensitivity to the treatment as the free drug [14]. In contrast, resistant cells treated with doxorubicin-loaded poly(alkyl cyanoacrylate) (PACA) nanoparticles showed a much higher sensitivity to the drug, relative to the free drug [10,11,14,15]. The sensitivity of the resistant cell lines even reached the level of sensitivity of the parent sensitive cell lines suggesting that the PACA nanoparticles can totally overcome the resistance mechanism. This was actually observed with different cell lines in which the resistance mechanism was only due to the presence of the P-gp, i.e. MDR-1 type [10–13]. This quite surprising result raised the issue of the specificity of the PACA nanoparticles. Different approaches have been developed to investigate the mechanism by which doxorubicin-loaded PACA nanoparticles overcome resistance to doxorubicin in the resistant cell lines. The degradation of the carrier was shown to play a key role in the mechanism of action, as was the requirement of direct contact between the colloidal carriers and the cells [15]. For instance, the intracellular concentration of doxorubicin in resistant cells is considerably increased by co-incubating doxorubicin with the degradation products of PACA nanoparticles. Moreover, it has been shown that poly(cyanoacrylic acid) resulting from nanoparticle degradation can form an ion-pair with doxorubicin [16]. In the same way, the sensitivity of the resistant cells to doxorubicin could only be increased when the nanoparticles were co-incubated with the cells. This increase in sensitivity was not observed when the nanoparticles were incubated in a separate compartment from the cells grown in a transwell culture chamber [15]. In contrast to what was initially believed, the endocytosis of the doxorubicin-loaded PACA nanoparticles is not required to enhance the sensitivity of the resistant cells to doxorubicin. Thus, intracellular concentrations of doxorubicin were found to be identical when working in the presence and in the absence of an endocytosis inhibitor like citochalasin-B [17]. The mechanism proposed to explain the ability of doxorubicin-loaded PACA nanoparticles to overcome the resistance to doxorubicin in resistant cancer cells is based on the adhesion of the nanoparticles to the cell surface. Adhesion is followed by the simultaneous release of the drug and nanoparticle degradation products that combine as an ion-pair able to cross the cell membrane without being recognized by the P-gp [15,18]. This mechanism, illustrated in Fig. 3, supposes that the nanoparticles fulfill three requirements: (1) adherence to the cell surface, (2) simultaneous nanoparticle degradation and release of drug, and (3) ion-pair formation of the degradation product and the drug. This is probably the reason why, to date, only PACA nanoparticles fulfill these requirements and overcome the resistance caused by the P-gp. Other types of nanoparticles that were tested failed to overcome P-gp-mediated MDR because they displayed inappropriate drug release, degradation kinetics or counter-ion size which could limit their diffusion across the cell membrane [13,14]. Strategies based on the idea of masking the positive charge of doxorubicin also failed, leading to marginal antitumor activity or to the use of non-biodegradable nanoparticles that are of limited use in vivo [13].

In all the studies described above, the P-gp remained active. Thus, more recent studies designed to further improve the efficacy of doxorubicin-loaded PACA nanoparticles in overcoming multidrug resistance were based on limiting the activity of the P-gp. This strategy also appeared to be an interesting alternative to promote the efficacy of doxorubicin-loaded nanoparticles in the case of a preliminary capture of the nanoparticles by macrophages. Soma et al. [19] suggested co-encapsulating doxorubicin and cyclosporin A within the same nanoparticles. Cyclosporin A is a chemosensitizing compound that can bind to P-gp and can inhibit the pump efflux activity. The nanoparticles

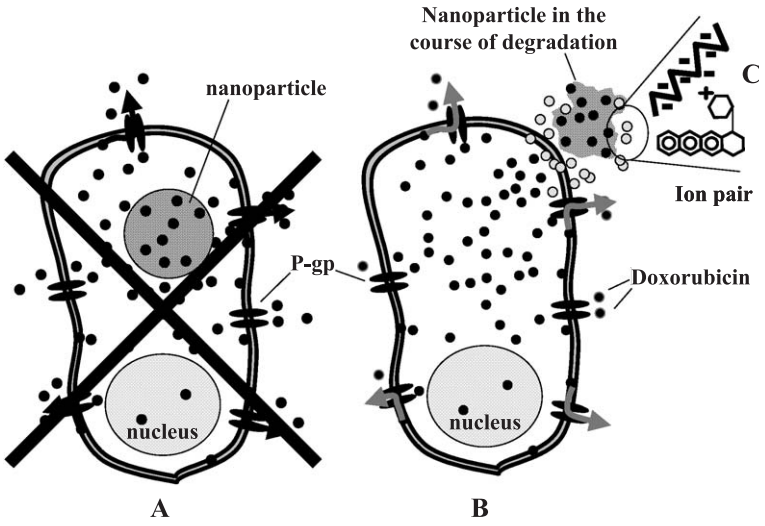


Fig. 3. Hypothesis about the mechanism of action of poly(alkyl cyanoacrylate) nanoparticles to overcome MDR at the cellular level. Drug-loaded nanoparticles are not endocytosed by the resistant cells (A) but adhere to the cell surface where they degrade and simultaneously release degradation products and the drug (B). The degradation products and the drug form ion-pairs (C) that can penetrate the cells without being recognized by the P-gp and, by this means, increase the intracellular concentration of anticancer drug in the resistant cells.

were prepared so that doxorubicin was incorporated within the core of the nanoparticles while cyclosporin A was located at the nanoparticle surface (Fig. 4). Using different formulations of the drug-loaded nanoparticles, it was shown in resistant cells and macrophage co-culture experiments that the association of both doxorubicin and cyclosporin A within a single nanoparticle elicited the most effective growth rate

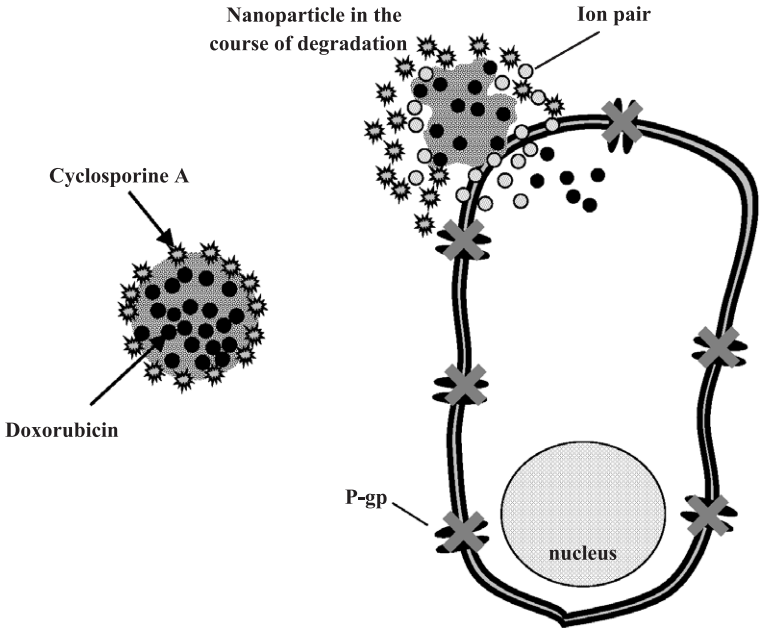


Fig. 4. Principle of the action of PACA nanoparticles co-encapsulating doxorubicin and cyclosporin A.

inhibition of the resistant cells. In such a co-culture, the doxorubicin-loaded nanoparticles by themselves can only partially overcome the MDR. The enhanced activity of the drug-loaded nanoparticles was interpreted as a result of a synergistic effect due to the rapid release of a high amount of cyclosporin A at the surface of the cell membrane, facilitating intracellular diffusion of doxorubicin. The association of cyclosporin A with doxorubicin nanospheres would also ensure that cyclosporin A reaches the same sites as the anticancer drug at the same time and also reduces its toxic side-effects.

Other strategies proposed to regulate the expression of the P-gp have involved using ribozymes [20] or oligonucleotides [21,22]. Because of the poor stability of these molecules in biological fluids, and because they poorly diffuse intracellularly, drug carrier systems were proposed. However, the results obtained were disappointing because of the long half-life of P-gp, making its down-regulation difficult [23,24].

## 3. The potential of nanoparticles to overcome multidrug resistance due to the more general problems of drug biodistribution

Potentially, nanoparticles can enhance the protection of anticancer drugs against biotransformation and rapid clearance from the body [25,26]. In addition, nanoparticles should have the proper biodistribution to target tumor tissue and tumor cells. With these objectives, studies carried out on PACA nanoparticles have focused on the customization of their surface properties. To date, three major approaches have been explored [7,27–30] (Fig. 5). In this paper, we will focus on studies conducted with PACA nanoparticles since only these nanoparticles have been shown to overcome multidrug resistance at the cellular level, as discussed above.

The biodistribution of PACA nanoparticles, first developed in 1979 by Couvreur et al. [27], has been shown to favor the organs of the MPS [31,32]. As such, these nanoparticles can be used to target anticancer drugs to the liver [25,26,33–35]. This biodistribution results from the natural host defense towards foreign particles involving a mon specific recognition phenomena; the latter is based on the opsonization of the nanoparticles by blood proteins and complement activation which lead to macrophages uptake [35,36]. It is interesting to point out here that Soma et al. [37,38] demonstrated that the MDR of P388 cells in culture was partially overcome after prior uptake of doxorubicin-loaded PACA nanoparticles by macrophages. This result is of particular interest in light of the results reported using doxorubicin-loaded PACA nanoparticles in the mouse liver metastasis model, in which it was found that the nanoparticles were taken up by the Kupffer cells [35].

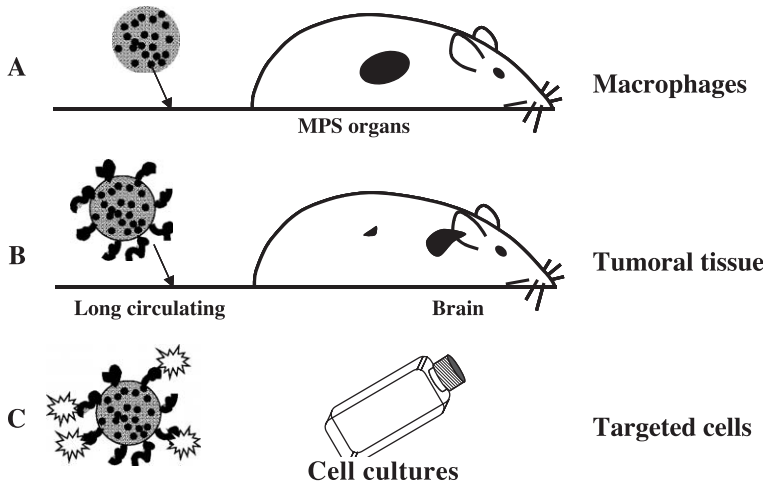


Fig. 5. State of the art regarding PACA nanoparticles with different surface properties. (A) Conventional nanoparticles, (B) PEG-coated PACA nanoparticles, (C) PEG-coated nanoparticles decorated with folic acid residues.

The second approach in the development of nanoparticles was aimed to modify the biodistribution of the carrier using poly(ethylene glycol) (PEG) as coating material grafter at the nanoparticle surface in order to reduce protein adsorption and complement activation [28,39–44]. PEG-coated PACA nanoparticles were prepared from a poly(PEG cyanoacrylate-co-hexadecyl cyanoacrylate) copolymer [45,46]. These nanoparticles circulated longer in the blood stream while their uptake by the liver was reduced [47]. They were found to accumulate into the brain [48,49] to a larger extent than other formulations, including the non-PEG-coated nanoparticles and PACA nanoparticles coated with poloxamer 908 and sorbitan 80 [50–53]. Particles were located in the ependymal cells of the chorionic plexuses, in the epithelial cells of pia mater and ventricules, and to a lower extent in the capillary endothelial cells of brain–blood barrier. This accumulation occurred without any modification of the brain–blood barrier permeability [48]. The concentration of PEG-coated nanoparticles in the central nervous system especially in the white matter was shown to be greatly increased in comparison to conventional non-PEG-coated nanoparticles. Recently, these nanoparticles were shown to accumulate specifically in glioma implanted into the brain. The accumulation was found to occur mainly in the tumoral tissue, while the amount of nanoparticles found in the adjacent healthy tissue and in the control hemisphere was much lower [54]. These nanoparticles were also found to improve the tumor targeting of recombinant tumor necrosis factor-α, leading to a higher accumulation of the drug in the tumor and to an increase in the antitumor activity [55,56]. In both cases, the increased accumulation of the drug in the tumoral tissue observed when the drug was administered in the form of PEG-coated PACA nanoparticles was attributed to the difference in the microvascular permeability between healthy and tumor tissue, combined with an increased circulation time in the blood stream.

To improve the specificity of the targeting of the PEG-coated PACA nanoparticles, the third approach considered grafting a molecular recognition moiety to the surface of the nanoparticles to achieve both the targeting of the cancer cells in the tumoral tissue and of the tumor after intravenous administration. Thus, in this case, the nanoparticles must show long circulating properties to reach the tumor tissue combined with specific recognition capacity of the targeted cancer cells once they have reached the tumor. With this aim, folic acid was conjugated to PEG-coated PACA nanoparticles [29]. The rationale behind the choice of folic acid as a targeting moiety is that folic acid binding proteins are frequently over-expressed on the surface of human cancer cells. The folate grafted PEG-coated nanoparticles showed a 10-fold higher apparent affinity for the folate binding protein than the free folate, as measured by surface plasma resonance. This increased apparent affinity was attributed to the fact that the particles represent a multivalent form of the ligand folic acid and can therefore display stronger interactions with the folate receptor [56]. Thus it could be expected that the folate decorated nanoparticles would also strongly interact with the surface of malignant cells on which the folate binding protein can form clusters; such binding can eventually promote endocytosis of the nanoparticles. The enhanced receptor-mediated endocytosis of the folate-decorated nanoparticles was clearly demonstrated using confocal microscopy. Indeed, only the cancer cell line over-expressing the folate binding protein showed intensive uptake of the folate-decorated nanoparticles. The cancer cell line that did not express the folate binding protein on the cell surface did not show any uptake of the same nanoparticles. In addition, none of these cell lines was able to internalize PEG-coated nanoparticles [57].

While progress in the development of PACA nanoparticles with different affinities now offers a choice between long circulating nanoparticles and targeted nanoparticles, the main limitation of these systems is the requirement of design and the synthesis of a new polymer for each type of nanoparticles to be developed. Thus, it was recently proposed to develop a simpler method allowing the preparation of nanoparticles using a single polymerization reaction and polysaccharides as biomimetic tools for modulating their surface properties. The approach is based on a new method of emulsion polymerization of alkyl cyanoacrylates initiated by a redox radical mechanism, leading in a single step to polysaccharide-PACA copolymers able to self-organize as nanoparticles [30,58,59]. The nanoparticles are composed of a degradable PACA core decorated with a polysaccharide brush exposed at the surface (Fig. 6). The suspension of nanoparticles is very stable, as evaluat-

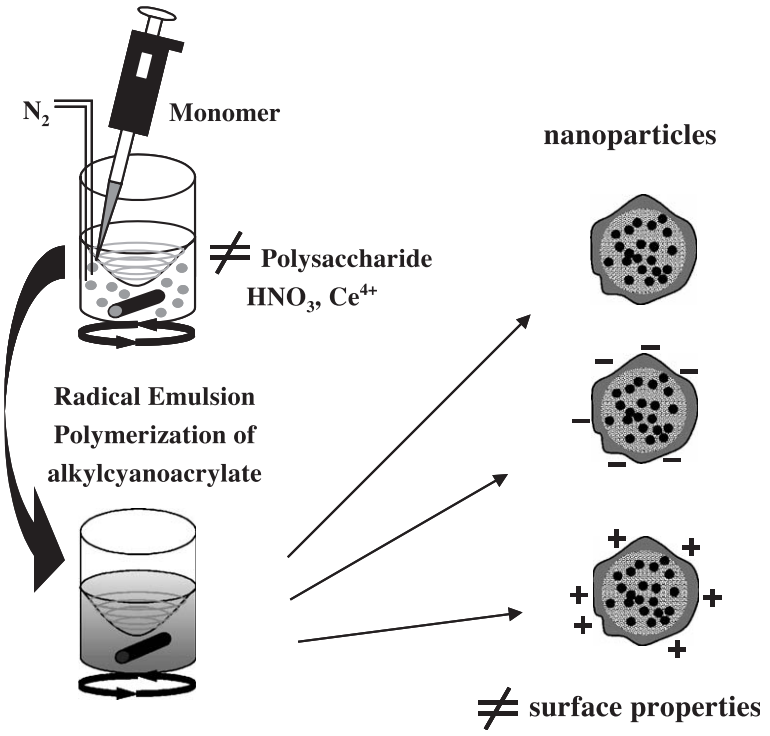


Fig. 6. Principle of the preparation of PACA nanoparticles decorated with a polysaccharide brush. The nanoparticle surface depends on the type of the polysaccharide used for the synthesis.

ed by size measurements, and can be lyophilized. The surface properties of these nanoparticles, including the zeta potential, complement activation and protein adsorption pattern, are defined by the nature of the polysaccharide used for the synthesis. The molecular weight of the polysaccharide also affects the nanoparticle surface properties when they are neutral. Indeed, short polysaccharide chains lead to incomplete shield of the nanoparticle core which contribute to the zeta potential of the nanoparticles. The biological activity of heparin was preserved at a level of 70% compared to the activity measured for a heparin solution. These nanoparticles offer new perspectives for the design of targeted nanoparticles using a biomimetic approach.

## 4. Conclusion

In cancer therapy, the occurrence of resistance phenomenon is a major obstacle for the treatment of tumors. PACA nanoparticles have been found to provide a useful alternative at a cellular level to overcome MDR mediated by the P-gp. These nanoparticles have been demonstrated to combine favorable drug release and biodegradation properties, while cell interactions of the carrier and its degradation products mediate the intracellular penetration of the drug. PACA nanoparticles have passed a clinical phase I trial [60] and have now reached the status of phase II clinical trials for resistant cancer.

In parallel with this work on resistant cells, progress has been made on the design of PACA nanoparticles with surface properties that allow better accumulation in tumor tissue after systemic administration. Although these nanoparticles have not been used until now in cancer therapy, it is likely that these types of constructions will be intensively investigated in the near future. It is also expected that future developments will concentrate on the design of nanoparticles loaded with emerging molecules such as taxol [61–63] and tumor necrosis factor [54,55] and

development of new anticancer strategies with the object of overcoming MDR.

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