Hyaluronic acid for anticancer drug and nucleic acid delivery

Hyaluronic acid for anticancer drug and nucleic acid delivery

Franco Dosio[1*], Silvia Arpicco[1], Barbara Stella[1], Elias Fattal[2,3]
[1] Università degli Studi di Torino, Dipartimento di Scienza e Tecnologia del Farmaco, v. P. Giuria 9, 10125 Torino, Italy
[2] Université Paris-Sud, Faculté de Pharmacie, Institut Galien Paris-Sud, LabEx LERMIT, 5 rue J.B. Clément, 92296 Châtenay-Malabry Cedex, France
[3] CNRS, UMR 8612, 5 rue J.B. Clément, 92296 Châtenay-Malabry Cedex, France

ABSTRACT
Hyaluronic acid (HA) is widely used in anticancer drug delivery, since it is biocompatible, biodegradable, non-toxic, and non-immunogenic; moreover, HA receptors are overexpressed on many tumor cells. Exploiting this ligand-receptor interaction, the use of HA is now a rapidly-growing platform for targeting CD44-overexpressing cells, to improve anticancer therapies. The rationale underlying approaches, chemical strategies, and recent advances in the use of HA to design drug carriers for delivering anticancer agents, are reviewed. Comprehensive descriptions are given of HA-based drug conjugates, particulate carriers (micelles, liposomes, nanoparticles, microparticles), inorganic nanostructures, and hydrogels, with particular emphasis on reports of preclinical/clinical results.

Keywords: hyaluronic acid, drug delivery systems, nanotechnology, CD44, conjugates, anticancer agents, liposomes, micelles, microparticles, hydrogels

*Corresponding author. Franco Dosio, Dipartimento di Scienza e Tecnologia del Farmaco, v. P. Giuria 9, 10125 Torino, Italy . email franco.dosio@unito.it, tel +390116706661, fax +390112367697

Authors' email address: Silvia Arpicco, silvia.arpicco@unito.it; Barbara Stella, barbara.stella@unito.it; Elias Fattal, elias.fattal@u-psud.fr

Contents

Abbreviations: ADH, adipic acid dihydrazide; AUNPs, gold nanoparticles; BA, butyric acid; CDDP, cisplatin; Cdots, nanodots; CHI, chitosane ; CNTs, carbon nanotubes; CPT, camptothecin; CSCs, cancer stem cells; DiPhPE, diphytanoyl-*sn*-glycero-3-glycerophosphatidyl ethanolamine; DL, drug loading (%w/w) = (mass drug in system/whole mass of system) *100; DLPE, 1,2-dilauroyl-*sn*-glycero-3-phosphatidyl ethanolamine; DMPE, 1,2-dimiristoyl-*sn*-glycero-3-glycerophosphatidyl ethanolamine; DOCA, deoxycholic acid; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; DOX, doxorubicin; DPPE, dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine; DSPE, 1,2-distearoyl-*sn*-glycero-3-phosphatidyl ethanolamine; ECM, extracellular matrix; EDA, ethylene diamine; EDC, 1-ethyl-3-[3-(dimethylamino)-propyl]-carbodiimide; **GAGs, glycosaminoglycan clusters of particles**; GFP, green fluorescent protein; GO, graphene oxide; GQD, graphene quantum dots; GSH, glutathione; HA, hyaluronic acid/hyaluronan; HA-MNCs, magnetic nanoclusters coated with HA; HARE, hyaluronan receptor for endocytosis; HBR, retinoic/butyric HA ester; HHSCs, hydrophobized HA-spermine conjugates; HIFU, high-intensity focused ultrasound; HSOP, HA-ss-(OA-g-bPEI); HYALs, hyaluronidases; ICAM-1 intracellular adhesion molecule-1; LYVE-1, lymphatic vessel endothelium

receptor-1; MSNCs, mesoporous nanocapsules ; MSPNs, mesoporous silica nanoparticle; MTD, maximum tolerated dose; MTX, methotrexate; MWCNTs, multi walled CNTs; NHS, *N*-hydroxysuccinimide; OVA, ovalbumin; PDT, photodynamic therapy; PE, phosphatidylethanolamine; PEG, polyethylene glycol; PEI, polyethylene imine; PHis, poly(L-histidine); PLGA, poly(lactic-*co*-glycolic acid); PTT, photothermal therapy; PTX, paclitaxel; PVA, poly(vinyl alcohol); QD, quantum dots; RHAMM, receptor for hyaluronan-mediated motility; SA, sodium alginate; SAL, salinomycin; SCCHN, human squamous cell carcinoma of the head and neck; SNP, silica solid core-shell nanoparticles; SNT, tubular shaped silica particles; SPIONs, superparamagnetic iron oxide nanoparticles; SPR, surface plasmon resonance; SWCNTs, single walled CNTs; TLR-4, tool-like receptor-4; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand

1. Introduction

Hyaluronic acid (HA) is the main component of the extracellular matrix (ECM) and is ubiquitously distributed in vertebrate tissues. Over half of the total body HA occurs in the skin [1, 2] **where it plays a structural role that depends on its unique hydrodynamic properties and its interactions with other ECM components. Conversely, HA has an instructive, cell signaling role during dynamic cell processes such as morphogenesis [3], inflammation [4], wound repair [5, 6], and cancer [7], wherein HA–receptor interactions are activated and collaborate in driving numerous signaling pathways [8, 9].**

HA is a nonsulfated glycosaminoglycan, comprising a relatively simple linear structure of alternating units of D-glucuronic acid and *N*-acetyl-D-glucosamine, linked via β-1,3- and β-1,4-glycosidic bonds (Figure 1). This chemical structure is fairly regular, with the exception of occasional deacetylated glucosamine residues [10]. It is synthesized by three transmembrane hyaluronan synthases (HAS1, HAS2, and HAS3) on the inner surface of cell membrane, and secreted in the ECM. These enzymes mediate the transglycosilation of D-glucuronic acid and *N*-acetyl-D-glucosamine, using their activated nucleotide sugars, uridine-5'-diphosphate-D-glucuronic acid, and uridine-5'-diphosphate-*N*-acetyl-D-glucosamine, as substrates [11-13]. The degradation of HA is due to hyaluronidases (HYALs), a family of enzymes that catalyze degradation of the β-*N*-acetyl-D-glucosaminidic linkages in the polymer [4]. The main uptake of HA from the blood takes place, in humans, in the liver endothelial cells, and HA has a half-life of about 2-5 min in the bloodstream, less than 1 day in skin, and 2-3 weeks in cartilage [14]. The daily turnover is extremely rapid, and in an adult of 70 kg amounts to 5 g.
The pK_a value of the carboxyl groups on the D-glucuronic acid residues ranges between 3-4, thus these groups are dissociated at physiological pH, and HA is a negatively-charged polymer associating with extracellular cations (Na^+) to form sodium hyaluronate. The term "hyaluronan" was introduced to encompass the different forms the molecule can take [15]. In mammalian organisms, native HA is present as a linear high-molecular-weight (HMW) polymer (10^6-10^7 Da), and this large molar mass gives it its unique physicochemical properties, and accounts for the important roles it plays in living organisms. HA is highly hydrophilic, and can absorb water and expand its solid volume by up to 1000 times, forming a very viscous and elastic gel with a large hydrodynamic volume [16].

The cluster of differentiation (CD) protein CD44 is the main hyaluronan binding receptor [17]. CD44 is responsible for the interaction between HA and the surface of specific cells. This interaction has been studied in depth, being involved in various cellular functions (both physiological and pathological processes). In particular, in normal physiology CD44 is involved in cellular adhesion processes (aggregation and migration), in inflammatory responses, and in repair systems. Conversely, the CD44 receptor is also associated with human cancer, being involved in tumor invasion and metastasis [7, 18]. CD44 is a family of transmembrane glycoproteins that are encoded by a single gene on the short arm of chromosome 11 [19, 20]. The phenomenon of extensive alternate splicing results in multiple variants of CD44 isoforms. The CD44 gene is composed of 20 exons, known as constant and variable

exons. The smallest CD44 isoform with no variable exons is called the CD44 standard form, or CD44s, while the others are known as variant CD44, or CD44v. It has been reported that CD44s is ubiquitous, while CD44v are mainly expressed in cancer cells [21]. CD44 is endogenously expressed in low levels in normal tissues [22], and its structure is modified in cancer cells [23]. Pathological conditions stimulate alternate splicing and post-translation modifications, producing diversified CD44 molecules with enhanced HA binding, which leads to increased tumorigenicity [21, 24]. Thus, CD44v are up-regulated on the surface of cancer cells. A specific CD44v isoform, named CD44v6, has been identified as the major isoform of the receptor that is overexpressed in various types of cancer, but not in corresponding normal tissues [25]. A number of clinical studies have demonstrated the correlation between CD44v6 expression and tumor progression in different types of tumors [24, 26]. Additionally, many studies in recent years have identified the role of CD44 in a subpopulation of tumor cells having self-renewal capacity, the so-called cancer stem cells (CSCs) or tumor initiating cells [27].

Several other HA-specific cellular receptors have been identified, such as the receptor for hyaluronan-mediated motility (RHAMM), recently designated as CD168 [28, 29]. This receptor mediates cell migration and proliferation; as is the case for CD44, it may be expressed in multiple splice variants. RHAMM is poorly expressed in most normal tissues, while its expression is increased in human tumors [30]. High expression of RHAMM is associated with metastases [31], and predicts shorter patient survival rates [32]. **Additionally, HA also interacts with HARE (hyaluronan receptor for endocytosis), LYVE-1 (lymphatic vessel endothelium receptor-1), and ICAM-1 (intracellular adhesion molecule-1), SHAP (for serum-derived hyaluronan-associated protein), Brevican and Neurocan (brain and nervous tissue-specific HA and proteoglycan binding proteins), HABP1 (for hyaluronan-binding protein 1) and TLRs (tool-like receptors) all of which have specific functions [33].**

The several roles played by HA in cancer progression have been investigated in depth. It has been reported that HA accumulates in the stroma of different tumors and activates signaling pathways that modulate cell proliferation, motility and invasiveness [24]. HA modulates angiogenesis, which is involved in tumor growth and metastasis and also induces the epithelial-mesenchymal transition, which is involved in the initiation of metastasis [34]. Moreover HA concentrations are higher in malignant tumors than in corresponding benign or normal tissues and in some tumor types the high level of HA [35], or in more recent findings, of a fragmented HA is predictive of malignancy [36].

It is noteworthy that the size of HA affects its biological functions. High molecular weight HA (4-20 MDa), which is present in intact tissue, is biocompatible, biodegradable, non-toxic, and non-immunogenic polymer [16]. In the presence of HYALs [4], HA undergoes rapid metabolism, giving molecules of various sizes, ranging from oligosaccharides (o-HA; <4 kDa or <10 disaccharide units), LMW (4-100 kDa) and HMW (>100 kDa) with different biological activities. **It is significant that a progressive weight reduction of fragmented hyaluronan strongly stimulates inflammation and angiogenic processes, tumor invasion, growth, and P-glycoprotein-mediated multidrug resistance [37-40]. Conversely, o-HA has been reported to possess tumor suppressive ability [41]; however it is difficult to determine the MW threshold responsible for one or other of these effects [4].**

The potential applicability of nonimmunogenic HA as drug carrier for parenteral and non-parenteral delivery is testified by the rapidly increasing number of reports on this topic over the last 15 years (Figure 2). Different strategies have been developed, either associating HA to drugs forming conjugates to improve their solubility, stability and efficacy, mainly in the case of anticancer drugs, or producing hydrogels, for the local delivery of various drugs, including antitumoral agents. However, one of the main applications employs coating of nano- and micro-technologies with HA for the delivery of anticancer drugs and nucleic acids, such as DNA or small interfering RNA (siRNA). A variety of strategies using organic or inorganic-based particles and targeting the hyaluronan–CD44 system have been proposed [42].

In the present review data concerning the rationale of the systems from the chemistry standpoint, and the choice of active agents, will be highlighted. As *in vivo* studies are imperative for estimating the

clinical feasibility of a drug delivery system, particular emphasis will be given to approaches reporting preclinical/clinical data, and/or to those showing a progression in research during recent years.

2. **Rationale for the use of HA in drug delivery**

For biomedical purposes, HA is mainly produced by microbial fermentation; it can also be extracted from rooster combs and umbilical cords [43]. **HA depolimerization can be achieved in batch cultures through either by enzymatic reaction or physical or chemical degradations [44-46].**
HA can be linked chemically to drugs or to drug carriers. The formation of HA drug conjugates, or the association of HA to colloidal carriers such as micelles, or to nanotechnology-derived particles, give several advantages. The most significant advantage is the ease of associating drugs with the polysaccharide, either directly or through a drug carrier, thus solving any solubility problems. A second advantage concerns HA's biopharmaceutical properties: it has been suggested that, in many cases, HA might improve a drug's blood plasma half-life, slowing the clearance mechanism, and thus playing a similar role to polyethylene glycol (PEG) [47]. Thirdly, concerning anticancer therapy, the possibility of tumor targeting is a significant advantage. Thanks to their enhanced pharmacokinetic properties, some HA-conjugates or HA-drug carriers may encounter the well-known enhanced permeation and retention (EPR) effect, leading to improved drug distribution in tumor tissues [48, 49]. Moreover, since CD44 is overexpressed in tumor cells and, particularly, in cancer stem or circulating cells, drug selectivity versus target cells may be improved [24, 50]. The possibility of overcoming the multidrug resistance (MDR) effect, which is sometimes related to overexpression of the efflux transmembrane Phospho-glycoprotein (P-gp), has also been reported [51].
At high concentrations in solution, HMW-HA can form viscoelastic entangled molecular networks known as hydrogels, in which drugs can be loaded either by association or via covalent linkage [52]. These hydrogels can be used for local delivery of antitumor drugs. However, solutions of HA do not have long-lasting mechanical integrity, particularly in physiological conditions [53]: HA hydrogels can swell by water absorption, or shrink on degradation. Covalent crosslinking is thus necessary to impart stability and improve functality. Thanks to the versatility of HA, a variety of chemically-modified forms of this polysaccharide have been developed, for use as tissue repair and regeneration materials, and also for the delivery of desired molecules in therapeutics; in particular, this latter concerns anticancer agents.
The reactivity of HA, and the principal chemical techniques used in developing drug delivery systems, will now be briefly described. The carboxylic groups and the primarily hydroxyl groups provide appropriate sites for conjugation, and are the most widely used groups for chemical modification. Comprehensive reviews by Schanté *et al.* [54] and Collins *et al.* [55] provide a full description of the variety of chemical modification methods and synthetic routes to obtain HA derivatives.
The carboxylic groups are involved in amidation and esterification reactions, and the primary hydroxyl residues in ester or ether bond formation (Figure 1) The acetyl group may be enzymatically removed from the *N*-D-acetylglucosamine, making it a potential site for conjugation [56]. When carboxylate and hydroxyl groups are modified, multiple attachments occur, and the groups are randomly linked to the polysaccharide chain, whether they are drugs, lipids, or polymers. Especially when the carboxylate group is selected as bridging point, it is important to determine the degree of substitution (DS) (ratio between the number of substituted reactive groups and the number of repeating disaccharide units of the polysaccharide) in order to maintain HA's overall charge and targeting properties: it has been found that a DS ratio above 25% decreased HA's ability to target CD44 receptors [57].
Amidation in water with carbodiimides is one of the most widely applied methods for HA modification; the most widely used carbodiimide is 1-ethyl-3-[3-(dimethylamino)-propyl]-carbodiimide (EDC), owing to its water solubility. The active intermediate, obtained at acidic pH values, does not easily react with amines. Replacing reacting amines by hydrazides, which have much

lower pK_a values, higher coupling degrees can be achieved: one of the most widely used reactants is adipic acid dihydrazide (ADH) [58]. To obtain more stable and more hydrolysis-resistant intermediates, *N*-hydroxysuccinimide (NHS) or 1-hydroxybenzotriazole (HOBt) are also commonly used. The obtained active esters show great reactivity towards the amines [59].
The hydroxyl groups of HA are commonly transformed into ester derivatives, by reacting them with the corresponding anhydride [60]. Alternatively, acyl-chloride-activated carboxylate compounds can be grafted through ester bonds [61].
The terminal reducing end of HA, which can react as an aldehyde group, may be involved so as to achieve a 1:1 stoichiometric ratio between polymer and reacting molecule. This approach involves the reductive amination reaction, usually using sodium cyanoborohydride as reducing agent, with an amino group of the reacting molecule (Figure 1, B). Further aldehyde groups may be obtained by reaction with sodium periodate, which oxidizes the hydroxyl groups of the glucuronic acid moiety of HA to dialdehydes, thereby opening the sugar ring. However, this reaction leads to a significant decrease of HA's molecular weight (Figure 1, C).
Thanks to the high hydrophilicity of HA, chemical modification can be performed in water; however, in the aqueous phase, some reactions require acidic or alkaline conditions that might induce significant HA chain hydrolysis, or entail the use of reagents sensitive to hydrolysis. Alternatively, organic solvents, such as dimethylformamide or dimethylsulfoxide (DMSO), can be used but, in this case, the HA sodium salt must be converted to its acidic form, or to a tetrabutylammonium salt, to make it soluble in organic solvents. The use of dimethoxy-polyethylene glycol (dmPEG) to solubilize HA in DMSO has also been reported [62].

3. HA drug-conjugates

The approach of producing polymeric macromolecule–drug conjugates was first proposed by Ringsdorf for the delivery of small hydrophobic drug molecules to their sites of action [63]. These drug-delivery systems basically comprise a water-soluble polymer, bearing a number of drug molecules, covalently linked.
The main advantages of this approach are that is i) increases the water solubility of low soluble or insoluble drugs, and therefore enhances drug bioavailability; ii) protects drugs from deactivation and preserves their activities during circulation; and iii) most importantly, that it actively targets the drug specifically to the site of action.
The benefit of using HA to design produgs is that it can act both as a hydrophilic carrier, available in a variety of molecular dimensions, and as a CD44 ligand targeting moiety. However, polymer-drug conjugates do not exist in solution, as the Ringsdorf "washing line model" implies; particularly when hydrophobic drugs are employed, aqueous solution conjugates tend to form micelles, nanoaggregates, or even gels, with the drug in the interior and a hydrophilic HA shell layer. Many structures also dynamically change their conformations in solution, depending on local salt concentration, polyelectrolyte counterions, and local pH, and/or during degradation of the components [64]. In addition, because HA behaves as an acidic polyelectrolyte, it can form complexes with molecules possessing an appropriate basic group. Non-covalent interactions with some drugs are reported [65].
During the chemical synthesis of HA conjugates, selection of the hinge component joining the two building blocks is of paramount importance: the conjugate must remain stable *in vivo,* while providing efficient release of the active moiety when the target cell or tissue is reached. This is essential to improve the therapeutic index of the prepared conjugates [66, 67].
The first reports of drug-HA conjugates were published in 1996: Akima *et al*. [68] conjugated HA with a fluorescent probe, mitomycin C (MTC), or with epirubicin. In a metastatic lung carcinoma model, HA-MTC decreased the number of metastatic lung nodules compared with free drug, whereas HA-epirubicin showed no activity. The HA conjugates with antitumor agents developed in recent years will now be presented; their principal characteristics have been summarized in Table 1.

3.1 Butyrate

In the late 1990s, POLYtech Srl (now Sigea Srl), developed the derivative HA-butyrate (HA-BA) (Figure 3, A). Butyrate is a histone deacetylase inhibitor (HDAC) which, after conjugation with HA, shows increased *in vitro* cell growth inhibition and apoptosis, and resulted in a decreased tumor burden *in vivo* [69]. Conjugates with DS ranging from 0.1 to 2% were prepared and tested; the most potent compound *in vitro* was composed of HA with MW of 85 kDa and a DS of 0.2, corresponding to a drug loading (DL) of 4% w/w butyrate. Intratumoral treatment of xenografted lung carcinoma showed HA-BA to have marked efficacy on primary tumor growth and on Lewis lung metastasis formation [60]. Interestingly, fibroblasts that expressed CD44 receptors to an extent very similar to that of tumor cells (83%) were completely unaffected by scalar doses of the compound, suggesting that, in normal slowly-proliferating cells, i.e. fibroblasts, HA-BA was completely ineffective notwithstanding the expression of CD44 **[60].**

Different administration routes were also tested, but after intravenous injection (i.v.), in just a few minutes there was a substantial accumulation of ^{99m}Tc-labeled HA-BA in the liver (45% of the injected dose); this uptake was considerably lower when the conjugate was administered intraperitoneally (i.p.) or subcutaneously (s.c.).

Using the s.c. route, HA-BA was also tested on a TLX5 lymphoma model, which induces both ascites and brain metastases. Treatment for 7 days demonstrated a 69% reduction in tumor cells in the peritoneal ascites, although without any increase in survival time, probably due to the inability of HA-BA to cross the blood–brain barrier and thus affect the brain metastases [70]. Interesting results were also obtained in HA-BA-treated mice in which intrahepatic lesions had been induced by intrasplenic inoculation of aggressive Lewis lung carcinoma LL3, or of melanoma B16/F10 cells. Complete regression of the melanoma occurred and, with regard to LL3 cells, 86% of s.c or i.p. treated animals were free of macroscopically-detectable metastases [70]. Similar effects were obtained on a pancreatic cancer model, in which antiproliferative, proapoptotic, and antiangiogenic activities were observed [71]. Further development of HA-BA has been proposed, in which other small molecules, such as 5-aza-2-deoxycytidine 3, retinoic acid, or 1α,25-dihydroxyvitamin D, are simultaneously bound to the same HA backbone. These molecules act through different mechanisms, but their biological activity might be potentiated by the presence of BA [70].

Finally, an HA conjugate with retinoic and butyric esters (HBR, MW ~ 85 kDa) was proposed, [72] to overcome retinoic acid resistance in acute promyelocytic leukemia patients. The conjugate was had DS ratios, with butyric acid and with retinoic acid, of 1.0 and 0.028% w/w respectively. *In vivo* antileukemic activity was evaluated in mice xenografted with NB-4 or P388 tumor cells after i.p. administration of HA conjugate, and the results showed that HBR blocks cell growth, and provides a significant increase in overall survival [72].

3.2 Paclitaxel

Paclitaxel (PTX) is a powerful drug recommended for ovarian, breast, lung, bladder, prostate, and esophageal cancers, melanoma, and other types of solid tumors, as well as Kaposi's sarcoma [73]. However, PTX administration is problematic owing to its poor solubility and relevant side effects, and also due to the poor tolerability of Cremophor®, the excipient typically used in its standard formulation. For these reasons, conjugation with hydrophilic polymers has been attempted since the late 1990s. Two research groups, one at Fidia Farmaceutici S.p.A., and the second coordinated by G. D. Prestwich, have used similar approaches to produce and characterize interesting HA-PTX conjugates.

The conjugates prepared by Fidia Farmaceutici were obtained by linking HA (~ 200 kDa) to PTX through 4-bromobutyric acid [74]. The compound denominated ONCOFID-P, with PTX loading of

20% (w/w), was initially developed for treating superficial bladder cancer [74]. Biodistribution studies of a ^{99m}Tc-radiolabeled ONCOFID-P, administered by different routes (i.v., i.p., intravesical, or oral), [75] showed that, after i.v. injection, rapid and marked liver uptake (around 80% of the injected dose) occurred. In contrast, imaging of the bladder, abdomen, and gastrointestinal tract after i.p. or intravesical administration showed that the radiolabeled conjugate remained compartmentally confined to the cavities. On oral administration, the greater part of the ONCOFID-P remained within the gastrointestinal tract, apparently not undergoing degradation. The study authors concluded that these approaches may be of interest, respectively, for the loco-regional treatment of transitional bladder cell carcinomas, ovarian cancers, and gastric tumors [75]. ONCOFID-P was subsequently evaluated by i.p. administration in nude mice, on ovarian cancer targeting xenografted CD44 overexpressing cells OVCAR-3 and SKOV-3. The studies demonstrated high tolerability of ONCOFID-P, with a maximum tolerated dose (MTD) of 100 mg/kg of PTX equivalent [76, 77]. In addition, i.p. administration of ONCOFID-P was overall more efficient than free PTX. In a schedule of 100 mg/kg PTX equivalent, injected weekly, ONCOFID-P inhibited intra-abdominal tumor dissemination, abrogated ascites, and prolonged survival by 1.8 to 3 times compared to free PTX (at MTD of 20 mg/kg), and even produced remission in some animals.

Based on these promising data, phase I studies have been started to investigate the MTD and safety profile of ONCOFID-P, following i.p. infusion, in patients affected by intraperitoneal carcinosis in ovarian, breast, stomach, bladder, or colon cancers [78-80]. Although in 2009 a phase II study was initiated on marker lesions, in the intravesical therapy of patients with non-muscle invasive cancer of the bladder (EudraCT 2009-012274-13), no conclusive data are yet available.

The approach followed by Prestwich involved the presence of an adipic dihydrazide (ADH) spacer between HA (~ 11 kDa) and the PTX-NHS ester (Figure 3, B)[81]. Klostergaard and coworkers carried out *in vitro* and *in vivo* studies, on different cancer models, of a similar conjugate using a higher MW HA (40 kDa) and achieving a PTX DL of 15-20% (w/w). On a xenografted human ovarian carcinoma, SKOV-3ip, the single i.p. administration of this conjugate (180 mg/kg of PTX equivalent dose) induced a markedly reduced tumor burden, while multiple-dose regimens of Taxol® at 10 or 15 mg/kg (MTD) were essentially inactive [82]. More recently, the i.p. administration with metronomic dosing (i.e., the frequent administration of chemotherapeutics at substantially lower-than-normal doses) of HA-ADH-PTX on taxane-sensitive (SKOV3ip1) and resistant (HeyA8-MDR) ovarian cancer models in mice has been investigated. The study showed that this regimen (9 doses for 20 mg/kg PTX equivalent, compared to a single dose of 180 mg/kg) had a substantial antitumor activity against taxane-resistant ovarian carcinoma, likely via a predominant antiangiogenic mechanism [83].

When tested by i.v. administration on a human squamous cell carcinoma of the head and neck, orthotropic CD44-expressing human xenografts OSC-19, and HN5 [84], a growth reduction of 86.2% for HA-ADH-PTX in comparison with 63.8% in the group receiving PTX was observed. Interestingly, the study authors reported relatively poor efficacy for i.p. administration in the same models.

Taking into account the stability of the linkage between HA and the drug, two opposing approaches are possible. Xin *et al*. reported that a direct ester bond between carrier and drug was hard to hydrolyze. Thus, following experience on PTX prodrugs, amino acids (aa) were proposed as spacers between the two compounds: an aa was first linked via the carboxylic group to the 2'-hydroxyl group of PTX, and then conjugated with LMW-HA (9.8 kDa) using EDC/NHS condensation [85]. HA-aa-PTX prodrugs with higher DL (10-15% w/w) spontaneously assembled into nanoparticles/micelles with size range 270-280 nm, having neutral charge. Owing to better recognition by the esterase enzymes, the presence of an amino acid spacer accelerated both the hydrolysis rate and the release of PTX, compared to HA–ADH–PTX conjugates.

Direct conjugation of the 2'-hydroxyl group of PTX to the carboxylate groups of HA is not easily achieved, due to the steric hindrance of the backbone. However, using HA with MW 64 kDa, and EDC/NHS condensation, Lee *et al*. [62] obtained conjugates with a DL up to 10 % (w/w) that spontaneously assembled into micelles of 200 nm. The same procedure was recently employed using a LMW-HA (5 kDa), and the resulting conjugate was tested *in vitro* and *in vivo* on brain metastasis

mouse models [86]. This low mass was selected due to its ability to cross the blood-tumor barrier, and penetrate metastatic lesions. This conjugate, with a PTX DL of 8% (w/w), in aqueous solution led to the formation of two different particle populations, of 2–3 nm and 80 nm. With an i.v. administration schedule of 6 mg/kg (PTX equivalent), once a week for five weeks, the conjugate improved the standard chemotherapeutic drug's efficacy in a preclinical model of brain metastases of breast cancer (231-Br cell line). The promising results on brain metastasis were attributed to the small size of the particles, and to the ability of the HA conjugate to diffuse and accumulate in lesions more markedly than conjugates containing HMW-HA. In addition, this approach also seems to circumvent the blood brain barrier efflux transporter P-gp, enhancing cytotoxic activity within lesions. The study authors stated that the 'ultra-low' molecular weight HA used in the study meant that it retained the ability to be internalized into the cell by receptor-mediated endocytosis.

3.3 Camptothecin

Camptothecin (CPT) is a cytotoxic compound that inhibits the DNA enzyme topoisomerase I. CPT is poorly soluble in water, and tends to open the lactone ring to generate the water-soluble CPT carboxylate, which has low cell uptake and is thus much less effective. To improve the stability and solubility of the compound, CPT prodrugs (including topotecan and irinotecan) or analogs have been developed, and are now being used in cancer chemotherapy [87]. Fidia Farmaceutici SpA conjugated one of these analogs, SN-38 (7-ethyl-10-hydroxycamptothecin), the active metabolite of irinotecan, through a butyric spacer to HA; the resulting compound was named ONCOFID-S (Figure 3, C) The conjugate, prepared starting from HA with MW of 200 kDa with a DL of 8% (w/w), showed strong *in vitro* cytotoxicity against several different $CD44^+$ cell lines: colon adenocarcinoma, and gastric, breast, esophageal, ovarian, and human lung cancers [88]. *In vivo* evaluation was on a xenografted colon adenocarcinoma in syngeneic rats. ONCOFID-S was administered by intralesion injection, or i.p.. ONCOFID-S at a weekly dose of 2.5 mg/kg (0.2 mg/kg SN-38), given intralesionally, totally suppressed tumor growth, while the same protocol for SN-38 given i.v. (5.7 mg/kg) was ineffective. After i.p. injection, a seven times higher concentration of SN-38 was required to exert the same antitumoral effect as the conjugate. Immunohistochemical analysis revealed that CD44v6 was highly expressed both on subcutaneous tissue and on the cells constituting the intraperitoneal tumor tissue. Pharmacokinetic evaluation and tolerability studies, as for ONCOFID-P, demonstrated low local toxicity, even after the i.p. administration of an extremely high dose (100 mg/kg) in rats. Because SN-38 was poorly diffused into the systemic circulation, this approach provided the rationale for applying ONCOFID-S in the loco-regional treatment of peritoneal carcinomatosis [89].
Further updates on the anticancer activity of both ONCOFID-P and ONCOFID-S have recently been reported [90]: mice with peritoneal carcinomatosis from HT-29, MKN-45, and OE-21 cell lines (respectively colorectal, esophageal, and gastric adenocarcinomas) were treated by i.p. administration with HA-linked conjugates or free drugs. The results confirmed the enhanced activity of these formulations compared to the free drug, corroborating the validity of this strategy to improve the loco-regional treatment of peritoneal carcinomatosis.
Xu *et al*. obtained CPT conjugates through an ADH spacer using HA of different MWs (100 and 8 kDa) and a DL of 1% (w/w). The MW affected CPT's water solubility and stability, but only to a negligible extent its antitumor activity [91].
Finally, Yang *et al*. described a novel chemical approach, in which 5% of HA carboxylate was replaced by aldehyde groups, by reaction with tartaric acid dihydrazide and mild oxidation. CPT was linked to HA (7.5 kDa) through a self-immolating disulfide linker to produce self-assembling structures; a reactive cholesterol derivative was also added at the same time and with the same linkage [92].

3.4 Doxorubicin

Doxorubicin (DOX) is among the most effective chemotherapeutics used in treating cancers. However, its use can be severely limited by its renal, hepatic, and most importantly cardiac toxicity [93]. Several formulations have thus been developed to improve DOX's efficacy and reduce its toxicity, including by employing liposome technology (Myocet® and Caelyx®).
DOX has been coupled to HA (35 kDa) through an ADH linker, obtaining a hydrazone-acid-labile linkage (Figure 3, D) and DL values ranging from 5% to 15% (w/w) [94]. Extended in vivo evaluations (renal and cardiac toxicity, pharmacokinetics, tumor model) were reported. The peritumoral injection of 3.5 mg/kg equivalent DOX dose of HA-DOX led to efficient intralymphatic delivery, which delayed tumor progression by approximately 10 weeks, and increased the animals' survival in comparison to treatment with DOX given i.v.. Furthermore, histological studies demonstrated that the injection site was devoid of inflammation or necrosis after HA–DOX injection, in contrast to what is generally described for free DOX.
A comparison between non-releasable amide (by direct linkage to 3'-amino group) and an acid-sensitive hydrazone bond linkage, was recently reported [95]. Using HA of 150 kDa and a low DL (0.2%–0.3% w/w), self-assembly occurred, generating nanoparticles of broad size distribution (581–1600 nm). It is significant that, although the DOX conjugate with a stable amide linkage has been described as active, it has more generally been reported to be less active than hydrazone or ester linkages [96-98]. To allow the release of free DOX from a carrier after 3'-amine reaction, a protease-sensitive and self-immolating linker is commonly preferred [99].
To further modulate DOX release from the conjugate, HA-ADH (320 kDa) has been fortified with up to 0.7 % w/w of thiol groups. At exposure to the air, self-gelation in occurred in aqueous solutions [100]. The conjugated DOX was released from the gel substantially in double pH and reduction-responsive modes. This approach was proposed for the loco-regional treatment of peritoneal dissemination of ovarian or gastric cancers.
A synergistic combination of topoisomerase I and II inhibitors (CPT and DOX) both linked to HA was recently proposed [101]. HA (250 kDa) was sequentially derivatized with CPT and DOX, using the carbodiimide method, obtaining DL of 5.9 %mol CPT and 1.8 %mol DOX. Although not yet completely characterized, the CPT-HA-DOX conjugate, after 5 daily i.v. injections of 2 mg/kg CPT and 1.05 mg/kg DOX, was able to reduce the size of a 4T1 orthotopic breast tumor by 70% in a mouse model.

3.5 Cisplatin

Cisplatin (cis-diamminedichloroplatinum (II) or CDDP) is the first member of an important class of platinum-containing anticancer drugs, which also includes carboplatin and oxaliplatin. CDDP is used clinically to treat different types of cancers, including sarcomas, and cancers of the soft tissue, bone, muscle, and blood vessels [102]. While highly effective, the use of platinum derivatives is limited by their severe dose-dependent side effects, particularly nephrotoxicity, neurotoxicity, and myelosuppression. In order to circumvent toxicity and increase tumor delivery, several approaches to HA-CDDP conjugates have been proposed.
HA-CDDP conjugates were first considered, and studied in depth, by Laird Forrest's group. In this research CDDP was linked to the carboxyl groups of HA (35 kDa) using silver nitrate as activating agent; the optimal DL dose was 0.25% w/w. It was demonstrated that the intralymphatic delivery model (by s.c. administration) of HA-CDDP conjugate not only significantly increased drug concentrations in loco-regional nodal tissues compared to the standard CDDP formulation, but also exhibited sustained release kinetics. Increased uptake by the liver, spleen, and kidney was also observed [103]. Sustained release was also observed in a lung instillation protocol of HA-CDDP [104]. Concerning efficacy, a selective anticancer effect in an *in vitro* model of non-small-cell lung cancer overexpressing CD44 was reported for these conjugates [105].

Further, in a recent *in vivo* study, a significant improvement in HA-CDDP antitumor efficacy was reported, together with reduced toxicity, when the formulation was administered in locally-advanced squamous cell carcinoma of the head and neck (SCCHN) [106]. More recently, a comparison between i.v. and s.c. administration protocols of CDDP and HA-CDDP, in a melanoma cell line A-2058 xenograft model, was reported [107]. Only the peritumoral administration of HA-CDDP dosing slowed tumor growth, and it was thus proposed as a potential therapeutic option in treating certain types of melanoma.
The HA-CDDP conjugate was also tested on dogs affected by soft-tissue sarcoma [108]. Tumor growth remained stable for 96 h after the intratumoral injection of HA-CDDP. No systemic CDDP-induced toxic effects were detected in any of the 5 dogs studied. More recently, the same group reported *in vitro* and *in vivo* studies on $CD44^+$ SCCHN, corroborated the targeting and slow release advantages for higher tumor Pt concentration of HA-CDDP administered s.c. [109]. Further studies demonstrated that the s.c. delivery of HA-DOX plus HA-CDDP conjugates (co-administered at 75% MTD, on a weekly schedule), in models of breast cancer, was significantly active, achieving complete pathologic tumor response, and showed decreased toxicity [110]. In the same trial, when injected systemically, the conjugate deposited in the liver and was cleared more rapidly than when it travels through the lymphatics.
Production of prodrugs that can release the active species inside the cell is a promising approach to reducing the toxicity of Pt (II). The oxidized species Pt (IV) can be activated in the reducing environment of cancer cells, and converted to the active CDDP. Recently, [111] the synthesis has been proposed and the *in vitro* and *in vivo* characterization described of a HA-ethylenediamine (EDA) conjugate of Pt (IV) (Figure 3, E). Using a succinic spacer, a DL of 14% (w/w), as Pt (IV), was linked to a HA of MW 11 kDa. The conjugate spontaneously assembled into nanoparticles of ≈180 nm in diameter. Tolerability of HA-EDA-Pt (IV) conjugates in mice was enhanced 30-fold versus CDDP. Anticancer activity was tested on melanoma B16-F10 implanted s.c. with daily injections of 6 mg/kg Pt(IV) of conjugate until the end of the experiment. Marked tumor-mass reduction and increased survival were achieved. Unusually, biodistribution analysis found the kidney as the principal organ of clearance.

3.6 Miscellaneous

HA has been proposed as an alternative to PEG, to improve the pharmacokinetic properties of therapeutic proteins. As a proof of concept, conjugates with trypsin, epidermal growth factor, and interferon-α (IFNα) were obtained [112].
In an interesting study, the targeting ability of HA was exploited to develop a conjugate with ovalbumin (OVA), in order to stimulate a cytotoxic T lymphocyte (CTLs) immunological rejection of tumor cells presenting the foreign antigen [113]. HA (35 kDa) was linked to OVA through reductive amination (Figure 1, scheme B). *In vivo,* an antitumoral effect was obtained (on a mouse cervical cancer model, TC-1, implanted s.c., highly expressing CD44) on mice previously immunized with vaccinia virus encoding recombinant OVA. The conjugate was injected i.v. daily for five days, and although liver uptake was strong, a large number of CTLs appeared, and thus significant tumor regression occurred.
HA has also been proposed as hydrophylic carrier for quercetin (QT), a potential anticancer agent with poor solubility. HA-ADH-QT conjugates behaved as micelles of about 170 nm. When tested on the hepatoma H22 model, the HA-QT conjugate, administered daily i.v. for the duration of the test, showed stronger antitumor activity than the free drug [114].

Table 1. Summary of the principal HA-drug conjugates with preclinical/clinical data available.

HA MW (kDa)	Drug	Administration route	Disease	Pharmacokinetic/ Biodistribution	Tumor Model	Reference
85	butyric acid	i.t., s.c., i.p.	Lewis lung, melanoma	☑	NCI-H460, B16F10, MCa, LL3	[60, 70]
85	butyric acid , retinoic acid	i.p.	Leukemia	-	P388	[72]
200	paclitaxel	i.p., intravesical	Ovarian, bladder cancer	☑	OVCAR-3, SKOV-3, Phase II	[74, 76, 77]
40	paclitaxel	i.p., mtn	Ovarian cancer	☑	NMP-1, SKOV-3ip, HeyA8-MDR	[82, 83]
40	paclitaxel	i.v.	SCCHN	-	OSC-19, HN5	[84]
5	paclitaxel	i.v.	Brain metastasis, breast cancer	-	231Br	[86]
200	camptothecin	i.p.	Peritoneal cancer	☑	HT-29, MKN-45, OE-21, DHD/K21/Trb	[88, 90]
35	doxorubicin	s.c.	Breast cancer	☑	MDA-MB-468LN	[94]
35	cisplatin	s.c.	Breast cancer	☑	MCF-7, MDA-MB-231	[103]
35	cisplatin	i.t.	SCCHN	-	MDA-1986	[106]
35	cisplatin	i.t.	Melanoma	-	A-2058	[107]
35	cisplatin	i.t.	Sarcoma (dog)	☑		[108]
35	cisplatin/doxorubicin	i.t.	Breast cancer	-	MDA-MB-468LN	[110]
11	cisplatin prodrug	i.v.	Melanoma	☑	B16-F10	[111]
35	ovalbumin	i.v.	Myeloma	☑	TC-1	[113]
10	quercetin	i.v.	Hepatoma	☑	H22	[114]

HA, hyaluronic acid; i.t., intratumoral; s.c., subcutaneous; i.p., intraperitoneal; i.v., intravenous; SCCHN, human squamous cell carcinoma of the head and neck; mtn, metronomic therapy

4. Organic nanostructured materials

4.1 Micelles

Amphiphilic hydrophobized polysaccharide polymeric micelles have been extensively studied for the delivery of water-insoluble anticancer drugs, since they possess small particle size, core-shell structure, high solubilization capacity, good *in vitro* stability, passive targeting for the EPR effect, active targeting through linkage with a specific ligand, and prolonged circulation [115]. Moreover, thermo- or pH-sensitive components may be added to the self-assembling copolymer structure, to control drug release upon an external or endogenous stimulus. The hydrated outer shell is normally helpful to avoid unwanted uptake by the RES. Poorly-water-soluble drugs may be physically entrapped or covalently linked to the polymeric backbone, at concentrations exceeding their intrinsic water solubility, thus improving bioavailability, achieving sustained release, and reducing toxicity. Hydrophobized polysaccharide polymers can also form complexes with charged molecules, and thus transport peptides, proteins, or nucleic acids.
Many factors may influence the properties of micelles, including the ratio of hydrophilic to hydrophobic parts of the structure, particle size, and compatibility of the drug with the copolymer [116].
Although micelles may greatly increase a drug's water solubility, they present some limitations. The principal barriers are low drug loading and encapsulation efficiency, poor *in vivo* stability (due to dissociation in conditions of dilution below the critical micelle concentration, and to interactions with blood components). Thus, suitable copolymers with certain specific properties must be chosen to prepare multifunctional polymeric micelles, to overcome these limitations. Additionally, excipients may also be added to the formulation.
HA-associated micelles are particularly attractive, since in these carriers HA may be employed in a dual capacity: as the hydrophilic polymer forming the outer shell of the micelle, and as the ligand to achieve active targeting of the micelle to $CD44^+$ cancer cells. As will now be reported, thanks to the reactive groups in its structure, HA can be employed to construct complex systems, in which the drug and other segments can be covalently linked to the polymer (see also Table 2).
Several biomolecules have been tested as the hydrophobic moiety in these conjugates; the characteristics of a micelle may be controlled by varying the degree of substitution. HA has also been used to stabilize drug loading onto micelles made of different materials. However, an important limitation in the use of HA-micelles is their preferential uptake by the liver after *in vivo* administration [117].
HA may be linked via its carboxylic groups to the amino functions of poly(L-histidine) (PHis) (a pH-sensitive polymer with endo-lysosomal escape properties), forming a tumor-targeted copolymer (HA-PHis) that can self-organize into micelles and carry anticancer drugs, such as DOX with a DL of 4-6% (w/w) [118] (Figure 4). The resulting HA-PHis copolymer contained pH-responsive blocks in its structure, due to the protonation of PHis, enabling DOX to be released in a pH-dependent manner, exploiting the lower pH of cancer cells. This system was then further developed to overcome the multidrug resistance of DOX: DOX-loaded micelles were obtained starting from a pH-sensitive mixed copolymer, composed of HA-PHis and D-α-tocopheryl PEG 2000, an inhibitor of P-gp-mediated drug efflux. These mixed systems (having a DL of 10% w/w) displayed stronger cytotoxicity than HA-PHis micelles against drug-resistant breast cancer MCF-7 cells. *In vivo* assays in mice showed that binary micelles accumulated in both liver and tumor, the concentration in the latter gradually increasing over time [119].
Another stimulus-responsive micelle material was prepared by conjugating deoxycholic acid (DOCA), as amphiphilic moiety, to HA (Figure 4). In the conjugate, cystamine was coupled via amidic linkages to HA and DOCA, and was exploited as a bioreducible linkage to prepare PTX-loaded redox-sensitive HA-deoxycholic acid (HA-ss-DOCA) micelles. The degradation of these

carriers depends on the glutathione (GSH) activity on the disulfide bond, and on the fact that a GSH concentration gradient exists between the intra- and extra-cellular compartments. Moreover, the GSH concentration in cancer cells is at least 4-fold that of normal cells. PTX was physically entrapped at high DL (up to 34.1% w/w), and anticancer activity both *in vitro* and *in vivo* in mice was higher than that of insensitive micelles (HA-DOCA carriers lacking the disulfide bond). HA-DOCA micelles also showed decreased systemic toxicity compared with Taxol® [120, 121].

HA redox-sensitive micelles have also been used for co-delivery of a hydrophobic and a hydrophilic compound, namely PTX and aurora kinase A (AURKA) siRNA, for breast cancer treatment. In this example of combined drug/gene treatment, a self-assembling amphiphilic conjugate was designed to form cationic polymeric micelles, in aqueous solutions known as HSOP (HA-ss-(OA-g-bPEI); in this system, octandioic acid (OA) and branched polyethyleneimine (bPEI) were linked to HA through cystamine (Figure 4). OA and bPEI respectively formed the hydrophobic core and the cationic segment for the encapsulation of PTX (DL 13% w/w) and siRNA. PTX and siRNA were simultaneously delivered into MDA-MB-231 breast cancer cells via HA-receptor-mediated endocytosis, thus reaching the cytosol. The *in vitro* and *in vivo* antitumor efficacy of the HSOP micelle in mice was compared to that of single-drug-loaded micelles, and non-sensitive co-loaded micelles. The HSOP micelles were found to possess the highest antitumor efficacy. Concerning the biodistribution profile, 2h after i.v. administration into MDA-MB-231 tumor-bearing nude mice, HSOP micelles had accumulated in both liver and tumor, whereas after 24 h preferential tumor accumulation was observed [122].

siRNAs to suppress cyclooxygenase-2 (COX-2) gene expression, used as model system, were encapsulated into micelles formed by hydrophobized HA-spermine conjugates (HHSCs) (Figure 4). *In vitro* tests showed that the cytotoxicity of these micelles on human gastric adenocarcinoma SGC-7901 cells was lower than that of siRNA complexes with PEI and Lipofectamine®, because of the negatively-charged HA, which reduced the positive charge density of the complexes. The results showed that siRNA/HHSC-1 (the complex with the lowest degree of substitution of spermine) was preferentially internalized into $CD44^+$ SGC-7901 cells (HA receptor-mediated endocytosis) by caveolae-mediated endocytosis, via nonacidic and nondegradable intracellular compartments [123].

Attention has also been paid to the chemical stability of drugs encapsulated in HA micelles. In particular, it has been reported that PTX may even change its phase (from crystalline to amorphous) when it is incorporated into drug carriers [124]. The physical and chemical structure of PTX was thus analyzed after its incorporation into micelles, obtained from HA hydrophobized by coupling it with C6 or C18:1 acyl chains. The results showed that PTX in HA micelles (DL 2-14% w/w) changed its form from crystalline to amorphous, and then isomerized; HA micelles containing PTX isomer were more strongly cytotoxic than the same micelles loaded with non-isomerized PTX [125].

Phospholipids are biodegradable and biocompatible components normally used to prepare liposomes but that can be also employed for the core part of micelles. HA, activated with EDC/NHS, have been linked to phospholipids (1,2-dimiristoyl *sn*-glycero-3-phosphatidylethanolamine (DMPE) and 1,2-distearoyl-*sn*-glycero-3-phosphatidyl ethanolamine (DSPE) via an amidic bond, obtaining micelles that can incorporate PTX (Figure 4). The resulting HA-DMPE and HA-DSPE micelles showed prolonged drug release *in vitro*. Differential scanning calorimetry studies again revealed that PTX had been converted into its amorphous form inside these micelles. After *in vivo* administration in rats, HA-DMPE and HA-DSPE micelles mostly accumulated in the liver, spleen, heart, and bladder [126].

PTX was also loaded into micelles formed by HA modified with 5β-cholanic acid (DL 7.7% w/w) for targeting CD44-overexpressing SCCHN model (SCC7 cell line). HA was conjugated to a derivative of 5β-cholanic acid (aminoethyl 5β-cholanoamide) through formation of an amide bond. When injected into SCC7 tumor induced mice, HA micelles accumulated in the liver, kidneys, spleen, lung, and heart, tumor accumulation increasing at the later time-points. *In vivo* studies also

showed that HA-5β-cholanic acid micelles inhibited tumor growth, with minimal side effects compared to free PTX [127].
PTX has also been successfully encapsulated into micelles, characterized by a dual-targeting strategy [128]: folic acid (FA) was conjugated to HA linked to hydrophobic octadecyl moieties (FA-HA-C18) to give self-assembling compounds [129]. In particular, grafting of the hydrophobic chains exploited the reaction between the HA carboxyl group and octadecylamine, while FA was linked via an ester bond to the 6'-hydroxyl groups of HA (Figure 4). PTX was physically incorporated into the hydrophobic core of the developing micelles, by an ultrasonic method in a dispersion of polymers. Nanosized micellar aggregates displayed DL values of up to 18% w/w. The dual targeting resulted in excellent uptake into cancer cells, by the clathrin- and caveolae-mediated endocytosis pathways. Moreover, these HA-PTX micelles were more cytotoxic for HA-receptor-overexpressing MCF-7 cancer cells than they were for HA-receptor-negative A549 cells. Further, dual-targeting micelles displayed a better MDR-overcoming performance, and higher PTX tumor accumulation, than did either single-targeting micelles or Taxol®, in MCF-7 tumor-bearing mice, although the micelles also showed high distribution in the liver, spleen, and lung [130]. The pharmacokinetic behavior of PTX-loaded HA-octadecyl micelles was studied in rats, showing that, after i.v. administration, the micelles possessed a longer elimination half-life in the blood circulation, and a larger AUC than Taxol® solution [131].
The grafting of HA to biodegradable copolymers to form CD44-actively targeted DOX-loaded micelles has been reported. In the first such study, HA was conjugated to hydrophobic poly(lactic-*co*-glycolic acid) (PLGA), using the PEG-assisted solubilization method in anhydrous DMSO; the terminal hydroxyl group of PLGA was conjugated to the carboxylic acid groups of HA via an ester linkage. The resulting amphiphilic copolymer self-assembled to form multi-cored micellar aggregates [132]. DOX was entrapped during micelle formation (DL 4-7% w/w, depending on the conjugate), and the carriers exhibited higher cellular uptake and greater cytotoxicity on $CD44^+$ HCT-116 cells than the free drug. In a similar approach, the carboxylic end group of PLGA was conjugated with hexamethylenediamine, and the terminal amino group was then coupled with the carboxylic function of HA. DOX-loaded micelles (DL 5-10% w/w) were prepared by the dialysis procedure [133]. In another study, HA was grafted onto hydrophobic polylactic acid (PLA) and amphiphilic PEG-PLA copolymers, to obtain a hydrophobic/hydrophilic balance appropriate to form DOX-loaded polymeric micelles in an aqueous medium. PEGylated micelles, which exhibited greater stability and had a higher drug-loading capacity (10.2% w/w) than non-PEGylated ones (4.8% w/w), were less sensitive to the adsorption of proteins from the culture medium. Moreover, as shown by cytotoxicity tests with PEGylated and non-PEGylated micelles, the presence of PEG on the carrier surface did not interfere with CD44 receptor recognition [134].
HA has been used as a tumor-specific shielding material on the surface of self-assembled cationic amphiphilic polymer micelles, formed of the hydrophobic all-trans-retinoic acid (ATRA) conjugated with a hydrophilic low-MW PEI. HA not only shielded the positive charges on the carrier surface, but also increased the micelle's stability. After systemic administration to CT-26 tumor-bearing mice, HA-shielded micelles accumulated selectively at tumor sites [135].
As reported above, HA has been used in micelle manufacturing processes not only as an amphiphilic hydrophobized polysaccharide, but also to stabilize drug loading. In a recent study, HA was used to load DOX onto amphiphilic apogossypolone (ApoG2, a compound that can induce autophagy and apoptosis in tumor cells) starch micelles; in particular, HA and DOX formed nanoparticles that were loaded by electrostatic absorption onto the ApoG2-starch micelles, forming mulberry-like dual-drug carriers (MLDC) (DL 13% w/w for both agents). This approach allowed the two drugs to be associated at optimal doses, with adjustable ratio and different release pathways. *In vivo* tests showed that these carriers had efficient active targeting, and accumulated in the tumor tissue when injected i.v. in PC-3 tumor-bearing mice. One-fifth normal dosage of the two drugs in MLDC exhibited significantly higher antitumor efficiency compared with a free-drug combination, or with two single-drug-loaded carriers given individually [136].

4.2 Liposomes

Liposomes have been investigated extensively as carriers for targeted drug delivery to specific cells, and major developments have been achieved in their pharmaceutical development, aimed at improving the efficacy of antitumor agents. Liposomal forms of DOX have been marketed, as Myocet® or Caelyx® (Doxil®), since they show improved anticancer efficacy compared to free DOX, giving enhanced drug accumulation in tumors [137-139]. Liposomes present several advantages as drug delivery systems. They can encapsulate both lipophilic and hydrophilic active drugs, respectively in the lipid bilayer and in the aqueous core. If they are made of natural compounds, they are highly biocompatible and biodegradable, and display low toxicity and immunogenicity [140, 141]. Further, they are very versatile vesicles, since their structure, particularly their surface, can be tuned to control their *in vivo* fate. Using cationic lipids in the liposome formulation affords complexation of nucleotides by ionic interaction, a strategy that has been extensively used for nucleotide delivery and in gene therapy [142].

Liposomes modified with HA have been investigated in depth, for drug targeting to CD44-expressing cells. Current work on the design of HA-liposomes has focused mainly on the selective delivery of antitumor drugs (small or large molecules) (see also Table 2).

Basically, there are two different preparation methods whereby HA can be inserted into the liposomal membrane. In the first, HA is linked to the surface of preformed liposomes by covalent conjugation. In this method [143], carboxylic residues of HA are pre-activated, by incubation with the condensing agent EDC in an aqueous acid medium. The activated HA is then added to the liposomal formulation at basic pH, when an amidic bond forms with the lipid amine groups. This method appears to yield coating efficiencies that afford efficient CD44-binding [144-151], which is desirable since a minimal amount of HA is necessary to target the receptors [152]. The drawback of this technique is that post-conjugation must be achieved on each batch, which is time-consuming, since the reaction lasts for more than 24 h [143]. In the second method, a HA-lipid is synthesized, the lipid anchor permitting insertion of the conjugate into the bilayer. LMW-HA can be linked to an aminated lipid, such as phosphatidylethanolamine (PE) [153], dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine (DPPE) [154], or diphytanoyl glycerophosphatidyl-*sn*-glycero-3-ethanolamine (DiPhPE) [155], by reductive amination (Figure 1, B). This gives a conjugate in which only one lipid molecule is linked to HA, and which will interact with other lipids within the bilayer structure. HMW-HA can be coupled to 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) by means of an amidation reaction, in which the amino group is randomly linked to the carboxylic residues of the polymer and then introduced into the liposome bilayer [156, 157]. This method affords faster preparation of HA-vesicles, since the conjugate can be synthesized and stored for further preparations. It also yields coating efficiencies that give efficient CD44-binding [155-159]. However, since most studies do not quantify the association efficiency of HA to liposomes, comparison of these two methods is difficult. Rather than being conjugated to phospholipids, HA may also be chemically coupled to other lipids, such as ceramide (CE) [158] or stearylamine [160].

The efficacy of HMW-HA bound to liposomes was first demonstrated by Peer and Margalit [161, 162]. Mitomycin C was encapsulated into these liposomes, and tested principally on BALB/c mice bearing C-26 solid tumor, and on C57BL/6 mice bearing B16F10.9 melanoma and D122 lung metastasis. Tumor progression, metastatic burden, and survival were superior in animals receiving MTC-loaded HA-liposomes. Moreover, with HA-modified liposomes, 30 times as much drug accumulated in the tumor as when the drug was administered in free form and 4 times as much as when delivered via unmodified liposomes. Interestingly, liver uptake was significantly reduced when the drug was delivered via HA-modified liposomes, which helped to reduce the subacute toxicity associated with MTC administered as free drug [161]. Similar results were achieved with different experimental models of tumors and HA-modified liposomes, in which MTC was replaced with DOX, thus demonstrating that the targeting process is carrier-specific rather than drug-specific [162]. In this latter study, the HA-modified formulation was compared to free DOX, DOX

encapsulated in unmodified liposomes, and PEGylated liposomes (Caelyx®). Drug accumulation in tumor bearing lungs, as well as key indicators of therapeutic response, such as tumor progression, metastatic burden, and survival, were superior in animals receiving DOX-loaded HA-modified liposomes, compared to controls [162]. A liposome formulation coated with HA was also developed for MDA-MB-231 targeted delivery of DOX and Magnevist (gadopentetate dimeglumine), a contrast agent for magnetic resonance imaging of breast cancer [158]. HA-modification reduced the liposomes' toxicity, increased serum stability, and promoted tumor accumulation. While plain liposomes immediately aggregated in the presence of 50% fetal bovine serum, the *in vitro* stability of HA-liposomes was maintained during 24 h. The *in vivo* diagnostic and tumor treatment capabilities of the formulation were evaluated in a MDA-MB-231 tumor-xenografted mouse model. The fluorescence signal in the tumor region, indicating the presence of the particles, was 2.6 times higher than with plain liposomes, and increased by up to 89% 2 h after injection, compared to the free drug distribution. The study authors attributed liposome accumulation in the tumor region to the serum stability of the particles and, more importantly, to its slow clearance *in vivo* and prolonged circulation in the blood stream [158].

In two other studies, [154] [159], HA-liposomes were prepared with LMW-HA, to target strongly CD44-expressing MiaPaCa2 pancreatic adenocarcinoma cells. The first study demonstrated a selective uptake of HA-liposomes by $CD44^+$ MiaPaCa2 cells, compared to $CD44^-$ VIT1 cells; the second study investigated cellular uptake and *in vitro* and *in vivo* anti-tumoral activity of HA-liposomes carrying gemcitabine. Both cellular internalization of liposomes carrying HA, and *in vitro* and *in vivo* sensitivity towards gemcitabine-containing HA-liposomes, were increased. In MiaPaCa2 cells treated with sufficient free HA to saturate the receptor, a significant reduction of the uptake of liposomes bearing HMW-HA was observed, suggesting that their internalization in MiaPaCa2 cells was mainly mediated by HA [159].

Dual-functional liposomes, with HA targeting and a pH-responsive cell-penetrating peptide, were prepared [163] for tumor-targeted drug delivery to hepatocellular carcinoma cells. HA was utilized to decrease liposome interaction with plasma proteins and, once at the tumor site, its degradation by HYAL exposed the R6H4 peptide. These PTX-containing liposomes displayed stronger cytotoxicity, efficient intracellular trafficking, and preferential tumor accumulation.

HA-liposomes have also been designed to deliver nucleic acids. A study [156] found that the transfection of DNA HA-lipoplexes on the CD44-expressing MDA-MB-231 cells was strongly inhibited by the anti-CD44 Hermes-1 antibody [164], but not by the nonspecific anti-ErbB2 antibody. Conversely, the transfection of MCF-7 cells, which express CD44 weakly, was not altered regardless of the presence of the Hermes-1 antibody. Another study tested the intracellular pathway for green fluorescent protein (GFP)-expressing DNA-cationic lipoplexes modified by HA. Cells were treated with 50 fold HA, which saturates CD44, or with HA-blocking CD44 antibody. In both cases, GFP expression decreased by 40% [157].

Other studies have used liposomes composed of neutral lipids to avoid the toxicity related to the cationic lipids. An anti Polo-Like Kinase 1 (PLK1) siRNA entrapped in such vesicles was found to reduce the protein level by more than 80% in an orthotopic model of U87MG tumor. In addition, survival of treated mice was also prolonged [165].

HA-based liposomes have also been designed containing miR-34a beacons (bHNCs) for the intracellular recognition of miR-34a levels in metastatic breast cancer cells. Upon blocking CD44 receptors with free HA, uptake of HA-coated liposomes was suppressed in both MCF-7 and MDA-MB-231 cell lines, although most markedly in the latter cell line. HA-liposomes afforded the targeted delivery of beacons via CD44 receptor-mediated endocytosis. *In vitro* and *in vivo* optical imaging, using bHNCs, also allowed the measurement of miR-34a expression levels, thanks to selective recognition of the beacons released from the internalized bHNCs [144].

Cell internalization of liposomes covered by HA has been studied in detail. Incubation of HA-liposomes with cells at 4 °C, a temperature that inhibits all active energy-mediated processes, significantly reduced the uptake in MiaPaCa2 cells, suggesting that HA-liposomes entered cells via

an endocytic pathway. These results were also confirmed in another study [166], that reported inhibition at 4 °C of HA-liposome uptake in A549 cells, compared to the uptake at 37 °C. This finding confirms that HA-liposomes are taken up inside the cells through an energy-dependent endocytosis pathway. The effects of several membrane entry inhibitors on the uptake of HA-liposomes have been examined by preincubating A549 cells with the inhibitors, before treatment with HA-liposomes. When blocking lipid rafts using methyl-β-cyclodextrin, uptake of HA-liposomes was reduced by nearly 45% compared to untreated cells, suggesting that HA-liposomes are taken up into the cells via lipid-raft-mediated endocytosis, which is cholesterol-dependent [145]. In a similar study, [154] MiaPaCa2 cells were pre-treated with increasing amounts of individual membrane entry inhibitors, before incubation with 4.8 or 12 kDa HA-liposome formulations. Flow cytometry analyses showed that methyl-β-cyclodextrin (MβCD) markedly decreased HA-liposome uptake, in a concentration dependent manner, while treatment with chlorpromazine, nystatin, and amiloride (respectively inhibitors of clathrin-mediated uptake, caveolae-mediated uptake, and macropinocytosis) did not alter cell fluorescence compared to controls. Regarding lipoplexes, incubation with the caveolae-inhibitor filipin of HMW-HA-bearing DNA lipoplexes reduced the transfection efficiency of A549 cells by 60%, using a plasmid coding for the GFP. Moreover, when genistein, another inhibitor of this internalization pathway, was used, GFP expression was lowered by 90% [157]. Both caveolin [167] and CD44 [168] are present in lipid rafts domains; it is therefore likely that inhibition of CD44 by an excess of HA or CD44 antibody blocks one of the pathways for caveolin phosphorylation, which is necessary to start caveolae-dependent endocytosis [169].
Regarding the pharmacokinetics of HA-liposomes, a systematic study has been carried out with three different HA polymer lengths (5-8, 50-60, and 175-350 kDa) and compared with that of PEGylated liposomes. Clearance of HA-liposomes was shown to be dependent on HA polymer length: the addition of HMW-HA to the surface of liposomes accelerated clearance of the particles from the blood compared to LMW-HA. This finding was related to the strong affinity of the particles for other HA receptors, such as HARE and LYVE-1, which are abundantly expressed in normal sinusoidal endothelial cells of the liver, spleen, and activated tissue macrophages [146, 170, 171], which are responsible for active clearance of liposomes from the circulation.
Lastly, HA-liposomes appear to be very safe, despite the fact that LMW-HA is considered to be proangiogenic, and to display proinflammatory properties. Indeed, a study that prepared liposomes with different MW HA reported that none of the vesicles had any apparent effect on the proliferation of highly CD44-expressing NCI/ADR-RES (for DOX-resistant cell line), TK-1, or RAW 264.7 cells. These nanocarriers did not appear to cause any macrophage activation or complement activation. Additionally, no cytokine induction was observed, regardless of the HA MW anchored to the surface of liposomes [148].

4.3 Nanoparticles

Nanoparticles form a very heterogeneous class of carriers, which have the submicron size in common but differ in composition, structure, preparation methods, and matrix degradability. They have been extensively studied for drug delivery and imaging, with several goals including controlled release, protection from degradation, enhanced bioavailability and better pharmacokinetic profile, passive and active targeting, and greater pharmacological efficacy. A nanoparticle-based PTX formulation is already on the market (Abraxane®); however, despite considerable research efforts in this field, drug-loaded nanoparticles are still associated with adverse effects, although they are less toxic than conventional therapies. In order to further enhance nanocarrier anticancer activity and reduce side effects, HA has been proposed as an active targeting agent [172]. Many studies report the use of HA to actively target nanoparticles towards CD44-overexpressing cancer cells, and/or to increase carrier blood-circulation half-life. The principal approaches to obtain HA-associated nanoparticles are described below; they have been subdivided

into two classes: HA-decorated nanoparticles, and HA-based nanogels. Table 2 summarizes the principal pharmacological characteristics of nanosystems described in the following pages.

4.3.1 HA-decorated nanoparticles

4.3.1.1 Polymeric nanoparticles

Ligand-directed polymeric nanoparticles have been explored in depth as drug carriers in anticancer therapy, since in some cases they exhibit enhanced in vivo stability compared to liposomes. They are also versatile carriers, thanks to the wide range of polymers and nanoparticle preparation methods. Moreover, many biodegradable synthetic and natural polymers are considered safe for pharmaceutical use [173]. Aliphatic polyester polymers, such as polylactide (PLA) [174], poly(lactide-co-glycolide) (PLGA), polycaprolactone (PCL), and poly(butylcyanoacrylate) (PBCA), have been conjugated to HA (Figure 5). HA-polymer conjugation has been achieved through direct procedures, or by adding linkers; a number of examples follow. Coupling procedures between PLGA and HA have been reported, in which suitable functional groups were added to the polysaccharide structure. Thanks to their amphiphilic properties, HA-PLGA polymers could then form nanoparticles with a core–shell structure, i.e., the hydrophilic HA domain formed the outer part of the nanoparticles, while the hydrophobic PLGA domain formed their inner-core. To this end, aminated 7.5 kDa HA (obtained by reduction and further coupling to hexamethylendiamine) was reacted with NHS-activated PLGA, and the amphiphilic conjugate was then used to form core-shell type nanoparticles containing DOX (DL up to 11.7% w/w). Anticancer activity assays on $CD44^+$ HCT-116 cells showed selective uptake via receptor-mediated endocytosis [175]. A similar approach was used to deliver docetaxel (DTX), a drug approved for the treatment of advanced or metastatic breast, head and neck, gastric, hormone-refractory prostate, and non-small-cell lung cancers. This in-depth investigation of HA-PLGA copolymers (HA MW 5.6-8.9 kDa, and DL up to 3.1% w/w) developed nanoparticles having longer circulation time in rats, and that in vivo efficiently delivered DTX to MDA-MB-231 breast cancer grown in mice [176].

In another study, HA-modified PLGA nanoparticles were prepared by adding longer linkers between the polymer chain and the HA backbone. DOX-loaded nanoparticles were formed by covalent conjugation of HA to PLGA via a diamine PEG-2kDa spacer. Both PLGA and HA (5.7 kDa) were first activated with EDC and NHS, then mixed with the diamine PEG linker. HA-PEG-PLGA nanoparticles were found to be more effective than PEG-PLGA carriers. Biodistribution studies showed high liver uptake, but also a significant concentration of HA-PEG-PLGA nanoparticles in the tumor mass of Ehrlich-ascites-bearing mice [177]. A similar approach was employed to load DOX into HA-PCL nanoparticles [178], and 5-fluorouracil (5FU), a pyrimidine analog used in treating several cancers, into HA-PEG-PLGA nanoparticles. Significant tumor uptake, and thus significant anticancer activity, were reported [179].

HA-PEG-PLGA nanoparticles have also been used to deliver letrozole, a potent aromatase inhibitor, to letrozole-resistant cells. HA nanoparticles were more efficient at inhibiting tumor growth in mice, reducing *in vitro* cellular and *in vivo* tumor aromatase enzyme activity, compared to the corresponding letrozole-PEG-PLGA nanoparticles or the free drug [180].

In a study designed to obtain redox-responsive DOX-loaded actively-targeted nanoparticles, a disulfide bond was introduced into HA-PLGA polymer prior to nanoparticle preparation, by adding cystamine to 7.5 kDa HA, which was then reacted with NHS-PLGA. DOX-loaded nanoparticles were then formulated (DL 4-7% w/w) and it was observed that, in the presence of GSH, drug release was facilitated [181]. A redox-responsive HA (6.4 kDa)-PLGA copolymer containing disulfide bond was also used to co-deliver DOX and cyclopamine (a primary inhibitor of the hedgehog signaling pathway of cancer stem cells) through water-oil-water HA-PLGA nanoemulsions. The unique interior structure of these carriers allowed two drugs, with different solubilities, to be efficiently encapsulated. Following this approach, PLGA was firstly functionalized with cystamine, and then reacted with NHS-HA. *In vivo* combination therapy

demonstrated a remarkable synergistic antitumor effect and prolonged survival, compared to monotherapy, using the orthotopic mammary fat pad tumor growth mouse model [182].
A reducible disulfide linkage was also used to obtain a cross-linked 12 kDa HA shell on DOX-loaded HA-PCL nanoparticles. The block copolymer was synthesized through a click-chemistry (azide-alkyne cycloaddition) approach: an alkyne group was introduced at the reducing end of HA, and then reacted with PCL-N_3. A disulfide linker was bound onto the HA backbone. After nanoparticle preparation, crosslinking of HA was achieved by the addition of a catalytic amount of dithiothreitol. Crosslinking of the nanoparticles markedly minimized the initial burst release of the drug. Moreover, HA-PCL nanoparticles displayed enhanced antitumor activity in tumor-bearing mice [183].
Nanocapsules with an oily core were prepared with HA-PEG-PCL to encapsulate DTX. The amine PEG/PCL was conjugated with HA (11.6 kDa) using a carbodiimide coupling reaction prior to nanocapsule formation. HA-PEG-PCL formed a thin polymer layer surrounding the inner core. DTX-loaded HA-PEG-PCL nanocapsules enhanced cellular uptake and anticancer activity, compared to PEG-PCL nanocapsules, in MDA-MB-231 cells [184].
HA was also grafted onto preformed PEG-PLGA nanoparticles loaded with SN-38, to target ovarian cancer. Both cellular uptake and cytotoxicity of HA-PEG-PLGA nanoparticles were higher in $CD44^+$ SKOV-3 and OVCAR-8 cell lines, than they were in $CD44^-$ cells [185].
Another approach to coating preformed polymeric nanoparticles has been reported: a mitoxantrone dihydrochloride-sodium deoxycholate complex and an efflux transporter inhibitor were embedded into nanoparticles prepared with Eudragit RL 100 and cetyl trimethyl ammonium bromide; HA was then deposited onto the carrier surface by electrostatic interaction; it gave the drug-loaded particles long-circulation properties in rats [186].
Another polymer suitable for drug delivery, PBCA, may be covalently derivatized with HA. In one study, nanoparticles were obtained through radical emulsion polymerization of *n*-butyl cyanoacrylate monomers, initiated by cerium ions in the presence of HA (18 kDa). HA-PBCA nanoparticles entrapping PTX were found to be more potent in suppressing S-180 tumor growth, following i.v. administration to tumor-bearing mice, than either PTX-loaded PBCA nanoparticles or PTX injection [187].
A multifunctional drug-delivery system based on 10 kDa HA-PBCA and D-α-tocopheryl polyethylene glycol succinate (TPGS) (a water-soluble derivative of natural vitamin E) was conceived to deliver morin hydrate, a naturally-occurring bioflavonoid identified in a number of fruits, vegetables, and herbs of the *Moraceae* family. The addition of TPGS during nanoparticle preparation increased the loading of morin hydrate (from 4.7 to 7.5% w/w) and enhanced its therapeutic efficacy *in vitro* and *in vivo* [188].
To obtain a photochemically-triggered cytosolic drug delivery system combined with tumor-targeting, acetylated 5.8 kDa HA was conjugated to poly((2-diisopropylaminoethyl)aspartamide), a pH-responsive moiety, and to the hydrophobic photosensitizer chlorin e6 (Ce6). Encapsulated DOX (DL 14% w/w) was released, as a consequence of protonation of the pH-responsive moiety inside the cell, and thus of the nanoparticles' disassembly. Low-intensity laser irradiation stimulated the free photosensitizer to produce reactive singlet oxygen, which released DOX into the cytosol of cancer cells, thus improving the anticancer efficacy both *in vitro* and *in vivo* in mice [189].
Finally, PLGA nanoparticles have been found able to load HA-drug amphiphilic derivatives: baicalein and PTX prodrugs were obtained by linkage to HA and to folic acid, respectively, and were then associated to the PLGA matrix during nanoparticle formation. This dually-targeted system showed enhanced synergistic anticancer effects, and overcame the MDR of PTX *in vitro* and *in vivo* [190].

4.3.1.2 Lipid nanoparticles

Lipid nanoparticles are widely employed in drug delivery, because of the biocompatibility of the lipid matrix [191]. HA has been linked to various lipid molecules. It may be conjugated prior to

nanoparticle formation, resulting in an amphiphilic molecule that self-assembles, producing a nano-sized carrier exposing HA moieties on the surface. Following this approach, HA (4.7 kDa) was conjugated via an ester linkage to ceramide, a component of cell membranes composed of sphingosine and fatty acid (Figure 5). The amphiphilic conjugate was formulated using Pluronic 85 as nanoparticles, to encapsulate DTX (DL up to 10.7% w/w). *In vitro* studies have shown that HA-CE nanoparticles enhance intracellular drug uptake in the $CD44^+$ MCF-7 cell line. Moreover, cytotoxicity tests showed that these carriers overcame MDR. *In vivo* studies after i.v. administration of DTX-loaded HA-CE nanoparticles, in MCF-7 tumor-bearing mice, confirmed their targeting ability for $CD44^+$ tumors [192]. In another study, DOX was successfully encapsulated into HA-CE nanoparticles for the treatment of melanoma (DL up to 7.8% w/w); DOX-loaded nanoparticles significantly inhibited tumor growth in a B16F10 tumor-bearing mouse model [193]. The HA-CE conjugate was then linked to PEG via the carboxyl groups of HA. PEGylation resulted in prolonged nanoparticle circulation in the bloodstream, and reduced the drug clearance rate, in an *in vivo* rat model [194]. In further research, HA-CE was used to encapsulate DTX-loaded PLGA nanoparticles. These hybrid lipid-polymeric nanoparticles were obtained by self-assembling HA-CE onto pre-formed DTX-PLGA nanocarriers. The mean diameter and zeta potential of the coated nanoparticles increased slightly (approx. 10%), confirming the surface deposition of HA-CE, which gave the nanoparticles *in vitro* and *in vivo* tumor targeting capabilities [195]. Other hybrid carriers have been produced for ginsenoside rg3 delivery, by combining HA-CE and lipids (phosphatidylcholine (PC) and DSPE-PEG) [196].

In another approach, HA (234 kDa) was used as drug carrier in HA-based nanoparticles; it was chemically linked to 5β-cholanic acid and PEG, to form tumor-targeted nanoparticles (P-HA-NPs); anticancer drugs (including DOX and camptothecin) were successfully encapsulated into the lipophilic inner core, and their release was increased in the presence of HYAL, which is abundant in the cytosol of tumor cells [197]. In the light of the encouraging *in vitro* cell-specific activity results and *in vivo* tumor-targeting characteristics, further studies were performed in order to encapsulate a number of different molecules, including for simultaneous tumor-targeted photodynamic imaging, and therapy with Ce6, or a near-infrared fluorescence imaging dye plus irinotecan [198, 199].

HA-NPs were also used to deliver siRNA. In this case, siLuc, a siRNA that targets the firefly luciferase gene, was used as a model compound; it was encapsulated in HA-NPs in which HA was modified with Zn_{II}–dipicolylamine complexes, which are highly selective for phosphate-containing molecules in aqueous solution. Unlike the cationic derivatives used to complex siRNAs, Zn_{II}–dipicolylamine-HA-NPs are expected to show lower toxicity and nonspecific accumulation, which are typically associated with polycationic-based formulations [200]. Moreover, to improve the *in vivo* stability of P-HA-NPs, photo-crosslinked nanoparticles loaded with PTX were prepared by UV-triggered chemical crosslinking with acrylate groups in the polymer structure. The release profile of PTX (DL 6% w/w) from nanoparticles was much slower than that from uncrosslinked P-HA-NPs. In addition, the photo-crosslinking procedure did not inhibit *in vitro* CD44 receptor-mediated endocytosis, and enhanced *in vivo* targeting ability in SCC7 tumor-bearing mice, as a consequence of the carriers' higher stability [201]. Alternatively, in an original approach, the stability of P-HA-NP was improved through mineralization: the DOX-loaded P-HA-NP surface was coated by means of the controlled deposition of inorganic calcium and phosphate ions, through a sequential addition method. Mineralization led to the formation of more compact nanoparticles; moreover, DOX (DL 8% w/w) was slowly released from the nanoparticles under physiological conditions, whereas its release rates were much higher in mildly acidic environments. DOX-loaded mineralized nanoparticles displayed excellent antitumor efficacy in SCC7 tumor-bearing mice, compared to both DOX-loaded bare nanoparticles and free DOX [202].

Reversibly-cross-linked polymeric DOX-loaded nanoparticles have been developed by conjugating, through a lysine, HA (35 kDa) with lipoic acid, a natural antioxidant produced by the human body. The lipoic ring is prone to ring-opening polymerization, forming a linear polydisulfide in the presence of a catalytic amount of dithiothreitol, under aqueous conditions; rapid drug release occurs

in the presence of 10 mM GSH. These multifunctional nanoparticles possess complete biocompatibility and biodegradability, and have the ability to uncross-link and become destabilized under cytoplasmic reductive conditions, resulting in an enhanced antitumor effect in $CD44^+$ drug-resistant human breast MCF-7 tumor xenografts in nude mice [203].

An interesting targeted co-delivery system was obtained from an amphiphilic 10 kDa HA-all-trans retinoic acid (HRA) conjugate, forming nanoparticles for potentially synergistic combination chemotherapy of PTX and retinoic acid. The HRA conjugate was obtained by reacting aminated retinoic acid with HA carboxyl groups. While all-trans retinoic acid was covalently linked to the HA backbone, PTX was encapsulated into HRA nanoparticles, which showed greater *in vitro* and *in vivo* anticancer activity, together with reduced toxicity, compared to the free drugs [204]. In a similar approach, another anticancer agent, gambocic acid (the main active ingredient of gamboge resin, which exudes from the *Garcinia hanburyi* tree in SE Asia) was encapsulated into HRA nanoparticles, achieving effective tumor-targeted co-delivery of gambocic acid and all-trans retinoic acid [205].

In another study, multifunctional nanoparticles, consisting of vitamin E analogues [α-tocopheryl succinate (α-TOS) linked to 7.8 kDa HA and TPGS], were prepared to encapsulate DTX (DL up to 7.3%). These nanoparticles displayed tumor-targeted delivery of DTX in a MCF-7/ADR xenografted nude mice models, overcoming MDR and enhancing the antiresistance therapeutic efficacy. It was expected that TPGS and α-TOS would serve, respectively, as an inhibitor of P-gp to overcome MDR, and to activate mitochondrial apoptotic pathways inside tumor cells [206].

An amphiphilic conjugate was obtained by reacting 10 kDa HA with aminated glycyrrhetinic acid (GA), a metabolite of the natural product glycyrrhizin exhibiting several pharmacological activities: it is anti-inflammatory, immune-modulating, and reverses MDR to anticancer agents. The HA-GA conjugate was formulated as PTX-loaded nanoparticles (DL 31.2% w/w) actively targeted towards liver tumors. HA-GA carriers displayed enhanced cytotoxicity on cells that overexpress both HA and GA receptors. *In vivo* assays showed it to accumulate in both tumor and the liver of MDA-MB-231 tumor-bearing mice [207]. This dually-targeted HA-GA system was also used to deliver DOX, in stimuli-responsive nanoparticles, to hepatocellular carcinoma cells. To this end, a reduction-cleavable disulfide bond was inserted between HA and GA; this meant that drug release was dramatically accelerated in the presence of intracellular levels of GSH [208].

The synthesis has been described of a series of functional variants of self-assembling CD44-targeting HA-based macrostructures, in which HA (20 kDa) was functionalized with lipids differing in carbon chain length, nitrogen content, and polyamine side chain, the goal being to achieve active targeting of siRNA to solid tumors. Using the EDC/NHS synthetic procedure, monofunctional and bifunctional fatty amines, multiple nitrogen-containing derivatives, and polyamines were coupled onto HA. The various derivatives showed differing abilities to form siRNA delivery systems, and to deliver it *in vitro* and *in vivo* [209]. A functional variant HA derivative, obtained by reaction with 1,8-diamineoctane, was used to encapsulate CDDP (DL 18-20% w/w) and to evaluate loaded nanoparticle biodistribution in mice, using various ultra-sensitive methods. The results showed that the most exposed organs were the liver, kidney, and spleen, while CDDP levels were very low in the heart and brain. Tumor, lung, and plasma accumulations were intermediate [210].

HA of different MW was associated to pre-formed DTX-loaded nanoparticles, prepared with cholesteryl hemisuccinate (CHEMS); this is an acidic cholesterol ester that self-assembles into bilayers in neutral or basic media, whereas in acidic conditions it undergoes a phase transformation, from the stable lamellar phase at neutral pH, to the unstable inverted hexagonal phase. Increasing the MW of HA from 35 to 1,490 kDa led to a significant increase in the size of HA-CHEMS nanoparticles, and a significant decrease in the zeta potential, as a function of the thickness of the HA layer. pH-responsive drug release occurred under different pH conditions. Compared to the commercial Taxotere®, *in vivo* HA-CHEMS nanoparticles exhibited higher tumor accumulation and antitumor activity in the MCF-7 mouse model [211]. The same research group applied a different approach to obtain DTX-loaded HA-CHEMS nanoparticles: HA (7.6 kDa) was first conjugated to

CHEMS, by an esterification reaction, after which DTX-containing nanoparticles were obtained by self-assemby of this amphiphilic derivative in an aqueous environment. Drug loading was higher than that of the previous HA-CHEMS nanoparticles (about 10% *vs.* 3% w/w). The degree of substitution of the hydrophobic moiety on HA greatly influenced the behaviour of nanoparticles: increasing the content of the hydrophobic group not only improved the stability of the nanoparticles in plasma, but also prolonged circulation time. Excellent tumor-targeting properties and efficient antitumor effects were obtained, with extremely low systemic toxicity [212].

Several studies report examples of HA-associated solid lipid nanoparticles (SLN). SLN are nanocarriers made from lipids that are solid at room temperature; they are used as an alternative to polymeric nanoparticles, thanks to their low toxicity, high stability, and scalability [191]. In most cases, HA has been associated to pre-formed cationic SLN by electrostatic interaction; the resulting formulation has given the loaded drug (generally a low-water-soluble molecule) stronger anticancer activity than when carried by non-coated SLN or as free drug. In addition, HA on the surface prolonged the half-life in the blood circulation. To give some examples: SLN coated with 3 kDa HA were tested for tumor-targeted delivery of vorinostat, a histone deacetylase inhibitor [213]; 300 kDa HA-SLN successfully delivered PTX inside melanoma stem-like cells [214]. A more complex SLN-based system has also been proposed: the surface of negatively-charged SLN was covered with a layer-by-layer coating of chitosan and 3kDa HA, to obtain hybrid nanoparticles containing DOX [215].

Nanostructured lipid carriers (NLC) are lipid nanoparticles, characterized by a core consisting of a mixture of solid and liquid lipids, in which the resulting matrix has a lower melting point than the original solid lipid, while still remaining solid at body temperature [216]. Compared to SLN, NLC generally have the advantage of offering increased drug loading and decreased drug leakage. Different approaches have been used to associate HA onto the surface of NLC: ionic conjugation [217]; grafting an alendronate sodium-11 kDa HA polymer by calcium-assisted mineralization [218, 219] to deliver irinotecan; and electrostatic interaction to co-transport a lipophilic prodrug of 5FU and CDDP [220] or PTX [221]. In these studies, HA decreased the carriers' toxicity, and increased NLC stability, tumor accumulation, and the *in vitro* and *in vivo* anticancer effect.

Gagomers (glycosaminoglycan clusters of particles; GAGs) are lipid-based non-liposome forming nanostructures that self-assemble into nanoparticle-like clusters, which are then covalently coated with HA by carbodiimide activation of carboxyl groups. HA is the main component of the GAGs surface; their inner structure can contain large quantities of nucleic acids and small (hydrophilic or hydrophobic) molecules. The HA coating naturally targets GAGs to cells expressing specific receptors and gives these particles stealth properties without triggering an immune response.

PTX-loaded GAGs with 500-1200 kDa HA were prepared and their structure and physicochemical properties were characterized. The cytotoxicity of PTX-GAGs was evaluated *in vitro* on the mouse colorectal carcinoma cell line CT-26, expressing CD44; it was demonstrated to be the same as that of free PTX. Pharmacokinetics, biodistribution and therapeutic properties of PTX-GAGs were evaluated *in vivo* after administration to CT-26 colon adenocarcinoma-bearing mice: the results showed a good safety profile, marked tumor accumulation, and antitumor potency superior to that of the FDA-approved PTX formulations (Taxol® and Abraxane®) [222].

GAGs with 750 kDa HA were tested *in vitro* on primary head and neck cancers and normal cells taken from the same patients; the results demonstrated a selective binding of GAGs to the cancerous cells, although the CD44 expression level was high in both normal and malignant cells. MTC was then encapsulated into GAGs and the cytotoxicity was tested on the same cell lines; the anticancer effect of MTC-GAGs on head and neck cancer cells was significantly higher than that of the free drug, whereas the viability of normal cells was not affected [223].

GAGs have been proposed as an alternative to cationic lipid-based formulations to efficiently deliver siRNAs and to induce gene silencing in cancer cells. siRNA against P-gp was encapsulated into GAGs coated with 700 kDa HA. After incubation with NCI-ADR/Res (NAR) cells that highly express P-gp, the results showed that siRNA-GAGs efficiently and specifically reduced mRNA and P-gp protein levels compared with controls [150]. In another approach GAGs were used to deliver siRNA into fluorescence-assisted cell sorting (FACS)-purified acute myeloid leukemia (AML) $CD44^+$ cells from two patients. *In vitro* analysis showed high transfection efficiency and co-localization of fluorescent siRNA and GAGs within the AML cell cytoplasm. Functional knockdown was detected using a qualitative polymerase chain reaction [224].

In order to bypass the drug resistance mechanism, DOX was encapsulated into GAGs bearing 700 kDa HA on the surface. *In vitro* the drug associated to the GAGs accumulated in the P-gp-overexpressing human ovarian adenocarcinoma cell line and dramatically decreased cell viability to a gretaer extent than either free drug or Doxil®; moreover, it overcame the P-gp-mediated resistant mechanism of these cells. A superior anticancer effect was also shown *in vivo*: in a mouse model of resistant ovarian adenocarcinoma where DOX associated to GAGs was shown to accumulate into the tumor more markedly than the free drug, and to significantly diminished the tumor growth compared with either free drug or the control treatments, without any observed clinical toxicity [225].

LMW-HA (<10 kDa) and HMW-HA (700 kDa) were conjugated onto the surface of lipid-based nanoparticles. The results showed that *in vitro* HMW-HA-conjugated particles had a higher affinity for $CD44^+$ B16F10 cancer cells than LMW-HA-linked nanoparticles. *In vivo,* neither types of carriers had any effect on triggering an immune response in healthy mice. The MW of HA influenced the nanoparticle circulation time and tumor targeting specificity, which were higher for HMW-HA-decorated carriers. MTX was then encapsulated into HMW-HA nanocarriers: after administration to B16F10 melanoma bearing mice these systems produced a better therapeutic outcome compared with any tested control [226].

4.3.1.3 Nanogels

Nanogels are nanosized networks with high water content; they belong to the class of soft drug delivery systems. Nanogels are usually biocompatible, stable and, as for hydrogels, they can be modified by linkage with various molecules. In nanogels, a hydrophilic polymer is either physically or chemically crosslinked [227]. Physically-crosslinked nanogels are the result of non-covalent attractive forces (hydrophobic or ionic interactions, hydrogen bonds) between the polymer chains; in chemically-crosslinked nanogels, the chains are covalently linked. The stability of covalently-bonded nanogels is due to crosslinkages that are obtained through different synthetic approaches [228].

With regard to HA-covered nanogels, HA-chitosan nanoparticles (HACTNP) containing 5FU have been designed to target colon tumors. 5FU-loaded chitosan nanoparticles were prepared by the ionotropic gelation method; using carbodiimide chemistry, their surface was coupled to the carboxylic group of HA (1.5 MDa), with the chitosan amine groups forming an amide linkage. HACTNP cellular uptake and cytotoxicity were evaluated *in vitro* on the human colorectal adenocarcinoma HT-29 cell line, which overexpresses the CD44 receptor. The results suggested that the 5FU-loaded HACTNP formulation had increased uptake compared to HA-uncoupled chitosan nanoparticles (CNTPs), and that it possessed enhanced cytotoxicity on the HT-29 cell line, compared with either CNTPs or free 5FU [229]. The same research group prepared HACTNPs containing oxaliplatin, to investigate anticancer activity and biodistribution *in vivo* after oral administration in a murine colon-tumor model. The biodistribution studies indicated that HACTNPs effectively reached the tumor, and were more effective in treating colon tumors than CNTPs or free oxaliplatin [230].

In a different approach, HA (170 kDa) and chitosan were used to form polyionic nanocomplexes, to co-encapsulate positively-charged DOX and negatively-charged microRNA-34a (MiR-34a), against triple-negative breast cancer. In this case, HA was not coupled onto the surface of the nanoparticles, but formed the matrix of the particles themselves, together with chitosan. The carriers were obtained by the ionotropic gelation method through a self-assembling procedure, and both DOX and MiR-34a were successfully associated through electrostatic interactions; the two drugs produced a synergistic antitumor effect *in vitro* and *in vivo* [231].

4.3.1.4 Complexes between nucleic acids and polycation polymers

HA was associated onto the surface of nucleic acid-polycation polymer complexes, to confer active targeting ability and to shield positive charges, thus increasing the stability and lowering the toxicity of nucleic acid vectors. To this end, HA (1000 kDa) was associated by electrostatic interaction to poly-L-lysine-graft-imidazole (PLI)-based polyplexes, containing Bcl-xL-specific shRNA-encoding plasmid DNA (HA/PLI/pDNA) for CD44-targeted gastric cancer therapy. Gel electrophoresis and cell viability experiments demonstrated that the ternary polyplexes had high stability and no cytotoxicity, thanks to the HA on the surface of the polyplexes [232].
In another approach, PEI was conjugated with HA to efficiently deliver MDR1 siRNA into OVCAR8TR (established PTX-resistant) tumors. The association of negatively-charged HA and PEI not only reduced the cytotoxic effects of PEI, through electrostatic neutralization of its positive charge, but also contributed to the formation of a protective hydrophilic surface. A HA-PEG derivative was co-formulated in these multi-component siRNA nanoparticles to give the carriers non-immunogenicity and stealth characteristics. HA-PEI/HA-PEG nanoparticles loaded with MDR1 siRNA efficiently down-regulated the expression of MDR1 and P-gp, inhibited the functional activity of P-gp, and subsequently increased cell sensitivity to PTX [233, 234].
PCL-graft-poly(*N*,*N*-dimethylaminoethyl methacrylate) is an amphiphilic cationic copolymer, which self-assembles into nanoparticles that efficiently condense DNA. Since these binary complexes are toxic and not stable in the blood, 46 kDa HA with grafted PEG side chains was synthesized to coat binary complexes by electrostatic interaction. Coating with HA-PEG improved the biocompatibility without reducing cell uptake or transfection efficacy, and resulted in higher accumulation and gene expression in mouse tumors [235].
An ATP-responsive system has been designed, in which a DNA scaffold was prepared by hybridizing an ATP aptamer and its cDNA, which has a 27-base pair with GC-rich motif, for loading anthracyclines. The DOX/Duplex was then mixed with protamine, to prepare a positively-charged DOX-loaded complex, onto which HA was deposited, and then crosslinked to give a nanogel. This system selectively releases the intercalated DOX via a conformational switch, when in an ATP-rich environment [236].

4.3.2 HA-based nanogels

Whereas, in the case of HA-decorated nanogels, HA is mainly located on the carrier surface, in HA nanogels HA forms part of the biocompatible and biodegradable matrix encapsulating the drug. The formation of nanogels can reduce the rapid degradation of HA in the blood.
PTX-loaded crosslinked nanoparticles were prepared with LMW-HA, using a desolvatation method with polymer crosslinking through glutaraldehyde. PTX molecules entrapped in the hydrophobic HA inner core did not significantly alter the average diameter of the HA nanoparticles. The resulting carriers had cytotoxicity similar to that of free PTX *in vitro,* with superior antitumor efficacy *in vivo* when administered by intratumor injection, for the treatment of mammary tumors in rats [237].
A methacrylation strategy has been proposed to functionalize 7 kDa HA with vinyl groups. The methacrylated HA was copolymerized with di(ethylene glycol) diacrylate to prepare enzyme-sensitive DOX-loaded crosslinked HA nanogels, in a completely aqueous medium. Lipase and

HYAL were found to de-crosslink the nanogels and release the drug. *In vivo* tests showed that HA nanogels enhanced DOX tumor accumulation, circulation time, and antitumor efficacy in H22 tumor-bearing mice [238].
HMW-HA (5000 kDa) with various degrees of acetylation was synthesized to prepare self-organized nanogels loaded with DOX. During a dialysis procedure, the acetyl groups formed the nanogel hydrophobic core, which may control pharmacokinetic properties, drug loading, and release. *In vitro* DOX-loaded nanogels showed selective cytotoxicity towards $CD44^+$ cancer cells [239].
o-HA (2 kDa) nanogels were obtained starting from the synthesis of conjugates between drugs (i.e. etoposide, salincomycin) and membranotropic cholesteryl-HA (CHA). The nanogel particles formed after ultrasonication, and demonstrated sustained drug release following hydrolysis of the biodegradable ester linkage. *In vitro,* after nanogel unfolding cholesterol moieties became anchored in the cell membrane, leading to more efficient drug accumulation in drug-resistant human breast and pancreatic adenocarcinoma cells, compared to nonmodified HA-drug conjugates [49]. CHA was also conjugated to curcumin; pharmacokinetic analysis revealed improved circulation parameters of CHA-curcumin after oral, i.p. and i.v. administration in mice; curcumin nanogels also efficiently accumulated in tumors *in vivo* and inhibited tumor growth [240].

5 Inorganic nanostructured materials

A number of types of nanoparticles composed of inorganic materials have been associated with HA. These include semiconductor nanoparticles or so-called quantum dots (typically cadmium sulphide and cadmium selenide), metals or metals oxides, and carbonaceous materials such as fullerenes and carbon nanotubes. No inorganic nanoparticle for drug delivery has yet achieved marketing approval; however, some promising clinical trials are producing robust data. Some examples are: NanoTherm®, an iron oxide nanoparticle coated with aminosilane, used in magnetic hyperthermia of glioblastoma [241]; gold nanoparticles (CYT-20000), manufactured by covalently linking TNFα and a thiolated PTX prodrug onto the surface [242].

5.1 Quantum dots

Quantum dots (QDs) are nanocrystals made of semiconductor materials (CdTe/CdSe, Cd_3P_2, InAs/ZnSe, and InAs/InP/ZnSe) with unique light-emitting properties. QDs, with emission wavelength 650 and 800 nm, have been tested *in vivo*. In order to achieve stable and improved molecular imaging, and to reduce their inherent toxicity, a coating may be applied around the QDs, using various ligands or organic compounds [243]. Only one research group has coated QDs with HA [244, 245]: the conjugation process involved HA with MW of 20, 234 and 3000 kDa, and linkage onto QDs was achieved through activated carboxyl terminals, using an ADH linker (Figure 3, A). An interesting correlation between ADH content (35 and 68 mol%), amount of HA, and liver uptake was observed. Using HA of 130 kDa, it was found that HA-QDs conjugated with a lower degree of derivatization, maintained sufficient binding sites for HA receptors, and accumulated principally in the liver, whereas those with higher ADH content were evenly distributed throughout the body [244].
Analogously, cysteamine-modified HA (7.5 kDa) was employed to coat QDs, through a convenient one-step reverse micelle method [246]. About 42% of HA carboxyl groups were converted into thiols, and that ensured strong binding to the QD surface, demonstrating long-term stability in buffers, excellent fluorescence stability in cell culture medium, and no significant cytotoxicity.
More recently, QDs were coated with HA (1000 kDa) linked to a melphalan derivative [247]. The drug release rate was much faster under acidic conditions (pH = 5.8) and in the presence of HYAL

(70%); *in vitro* tests showed rapid internalization (intense fluorescence) in $CD44^+$ MDA-MB-453 cells, compared to normal breast cells.

5.2 Metal (gold and iron oxide) nanoparticles

Gold nanoparticles (AuNPs) have a number of physicochemical properties that distinguish them from other inorganic nanocarriers, principally chemical inertness, simplicity of surface modification, and interesting optical properties. An increasing number of experimental studies concern AuNPs exploited for drug and gene delivery, immunotherapy, photo-ablation treatment, as well as for use as biosensors and in imaging [248].
Coating and targeting with HA has mainly been limited to imaging uses [249]. A hybrid nanomaterial was recently developed in which AuNPs were linked in a lattice of thiolated HA bearing pheophorbide-A (PheoA), a photodynamic agent. This approach successfully combined photothermal therapy (PTT, AuNPs), photodynamic therapy (PDT, PheoA), and a cancer-targeting agent [250]. Nanohybrids of 90 nm showed cytotoxicity only after laser irradiation. Photothermal images after i.v. injection and irradiation demonstrated pronounced tumor localization. *In vivo* studies on A549 cancer cells implanted subcutaneously showed that, when the tumors were exposed to i.v. administration of PheoA-HA/AuNPs every 2 days, for a total of 24 days, and concomitant laser irradiation, no tumor growth occurred throughout that period, and significant lesions were observed in the tumor tissues [250].
Other types of metal particles, such as iron oxide or superparamagnetic iron oxide nanoparticles (SPIONs), were associated to HA for imaging purposes; among anticancer agents, only DOX has to date been linked to HA–coated SPIONs (31 kDa). Through an ADH linker, DOX was conjugated to HA (31 kDa) and to amine-containing SPIONs (Figure 6, A). Nanoparticles having a solid core of 5 nm and a hydrodynamic diameter of 114 nm carried DOX at a DL of 2.1% w/w. Increased magnetic resonance imaging of cancer cells, combined with increased cytotoxicity compared to the free drug, were reported [251].
Another study described the preparation of PEI-stabilized iron oxide nanoparticles, coated with HA of two different MW (6 and 31 kDa) [252]. *In vitro* analyses demonstrated that the nanoparticles are specifically taken up by HeLa cells overexpressing CD44 receptors. In addition, the nanoparticles with HA of 31kDa had a better targeting ability than those with HA of lower MW. A biodistribution study in mice xenografted with HeLa tumor revealed good tumor targeting, although the liver and spleen with both types of nanoparticles took up most of the iron.
Different formulations of SPIONs with surface functionalized with HA (6.8 kDa), with or without PEG 10 kDa, have recently been compared in *in vitro* hyperthermia and *in vivo* MRI studies. The findings showed that HA-SPIONs had better T2 contrast efficiency, with a similar hyperthermia effect, compared to the particles containing PEG [253].

5.3 Carbon-based nanoparticles

Among the inorganic nanomaterials, carbonaceous nanomaterials are attracting interest due to their excellent mechanical, thermal, and optical properties. These characteristics may allow the development of promising platforms, combining diagnosis and therapy into one device. For instance, most of these nanomaterials possess strong absorption in the infrared or near infrared regions, which is useful for PTT. Some of them (carbon nanotubes or nanodots) can also produce fluorescence in the visible and infrared regions, for fluorescence imaging. In addition, carbonaceous nanomaterials are able to transform laser-irradiated energy into acoustic signals, making them promising agents for photoacoustic imaging. The properties of nano-carbon-based materials vary with the dimensions and shape of the particles. Depending on these parameters, they can be

subdivided into fullerenes, nanotubes, nanohorns, nanoonions, nanodiamond, nanodots, and graphene derivatives [254, 255].
The inert composition of these materials is highly beneficial, making them biocompatible and thus highly desirable for biological applications; however, since most pristine carbonaceous nanomaterials are highly hydrophobic (due to the sp^2 carbon structure), appropriate functionalization is necessary before they may be used in biomedical applications. The hydrophobic interaction, or π–π stacking, enables multiple copies of drugs or DNA/RNA molecules to be absorbed. Moreover, the addition of stimulus-responsive polymers (e.g., to light or heat) to carbonaceous nanomaterials can also be extremely useful for selective and controllable drug release.
However, the limited data yet available on toxicity means that further efforts are required to evaluate in depth not only the potency but also the *in vivo* fate of these formulations (e.g. biodistribution, elimination from the body), and a complete toxicology evaluation must be made, before they can be administered safely.

5.3.1 Fullerenes

The smallest known carbon-based nanostructure, buckminsterfullerene (C_{60}), has received considerable attention also in the oncological field, because it is regarded as being an excellent photosensitizer for use in PDT. Fullerenes possess almost 100% generation yield of $^1O_2$, and have attracted considerable attention as photosensitizers in antitumor applications; however, they can also act as "radical sponges", demonstrating anticancer properties in themselves [256].
HA may be a system of choice to improve the solubility in water and biological media of C_{60}. In a recent study, the preparation was described of hyaluronated fullerenes [121], produced in the presence of lithium hydroxide as catalyst. This reacted with the fullerene to break the π–π carbon bonds, after which it was combined with the HA hydroxyl group, yielding various forms of carbon–oxygen conjugates [257] (Figure 6, B). Using HA (4 kDa) within nanoparticles of 30–60 nm induced strong NIR fluorescence. However, fluorescence intensities gradually decreased as the degree of C_{60} substitution increased (from 0.05 to 0.78 C_{60} per one sugar unit of HA). After irradiation, the compounds were found to be cytotoxic to $CD44^+$ cells. After i.v. administration to mice bearing HCT-116 human colon cancer cells, C_{60}-HA effectively accumulated in the tumor tissue, although high fluorescent intensity was also noted in the liver. 24 h after injection of C_{60}-HA (0.05 C_{60} per one sugar unit of HA) and laser irradiation, significant tumor volume regression continued for 7 days.
The same research group recently proposed a double-targeting conjugate for C_{60}, in which HA and Hoechst 33258 (capable of detecting necrotic tumor cells) were linked [258]. *In vitro* phototoxicity and *in vivo* tumor inhibition both increased after multiple photodynamic treatments.
Although different products (PDT agents, DOXO, genes) have been described as being successfully delivered by C_{60}, no reports of HA-coated C_{60} loading drugs have yet been published.

5.3.2 Carbon nanotubes

Carbon nanotubes (CNTs) are graphite-like structures that are inert in nature. In terms of biomedical applications, CNTs have shown important potential in several areas, from tissue engineering to drug delivery [259].
Single-walled CNTs (SWCNTs) consist of a single graphene cylinder, with diameter varying between 0.4 and 2 nm, whereas multi-walled CNTs (MWCNTs) consist of two to several coaxial cylinders, each made of a single graphene sheet, surrounding a hollow core. The outer diameter of MWCNTs ranges from 2 to 100 nm, and their length from 0.2 to several μm.
With regard solely to CNTs as drug delivery agents, their major advantages are their inertness, their intrinsic imaging properties (fluorescence and Raman scattering), and their high surface area.

SWCNTs also exhibit strong absorbance in the near-infrared region, and generate significant amounts of heat upon excitation. This photothermal effect can induce the local thermal ablation of tumor cells. Further, this effect can also be combined with the concomitant release of CNT-loaded drug. Some progress in these techniques has been achieved in recent years, which have been shown to be feasible for clinical applications [260].

There are three ways in which the carrier can be loaded with drugs. Firstly, the drug can be chemically linked onto the surface of CNTs, either permanently or through cleavable linkers. Secondly, drugs that possess conjugated aromatic ring systems can be physically adsorbed onto the surface of CNTs, through non-covalent π–π and hydrophobic interactions. Thirdly, some drugs can be encapsulated within the interior cavity of CNTs.

Many anticancer drugs have been used with functionalized CNTs, exploiting different chemical linkages or entrapping the drug onto/inside the tubes; examples are epirubicin, DOX, cisplatin, methotrexate (MTX), QT, and PTX.

In the attempt to improve water solubility and stability, the effects of coating the surface of CNTs have been examined. SWCNTs functionalized by collagen, proteins, polymers, PEGylated lipids, or block copolymers, single-stranded DNA, Pluronic copolymers, and HA, have all been described [261].

The selectivity and targeting properties of CNTs can be improved by attaching ligands to their surface, for example folic acid, peptides, epidermal growth factor, or antibodies. HA has been exploited to increase the solubility of CNTs and to obtain an active targeting effect. Several strategies have been developed, whereby HA was covalently linked or adsorbed onto the surface of CNTs.

A “complete covalent” approach, in which HA bearing drugs was linked to SWCNTs, has been described. HA (5 kDa) was functionalized with amino groups at the primary hydroxyl groups, and then these were reacted with the carboxylic groups of SWCNTs. The free amino groups of the HA chains were then further functionalized with the anti-inflammatory drug ibuprofen, and with MTX [262].

The covalent linkage of HA onto MWCNTs, followed by adsorption of DOX, has also been described [263]. After oxidation of MWCNTs, the carboxyl functions were interchanged with amine groups, in excess 2,2'-(ethylenedioxy) bis(ethylamine) (Figure 7, A). HA of different MWs (120 and 5 kDa) were then condensed to amine groups, and the resulting HA-MWCNTs were successively loaded with DOX, obtaining a DL of 33% w/w. Biodistribution studies in the Ehlrich ascites tumor-bearing mouse model indicated that HA-MWCNTs were chiefly distributed in the liver, lung, and spleen, although to a lesser extent than occurred with uncoated nanotubes. LMW-HA coated MWCNTs accumulated in tumors with greater efficiency (5.79 and 3.18 times) than did free DOX and uncoated MWCNTs, respectively. The efficacy and toxicity were assessed in Sprague Dawley rats, with chemically-induced mammary tumors. A single dose of 5 mg/kg demonstrated slightly higher antitumoral activity than that of free DOX.

A similar approach has been described [264] in which HA-decorated SWCNTs were used to deliver hematoporphyrin monomethyl ether (HMME, Hemoporfin®), a photosensitizer that has been in clinical trials in China since the early 1990s; the goal was to obtain a nanoparticle PDT agent. Moreover, PTT-capable CNTs in the NIR region (808 nm), and PDT-capable HMME in the visible light region (532 nm), were administered simultaneously to reinforce the antitumor effect. A very high DL (70% w/w) was reported. The anticancer activity of these compounds was tested on mouse melanoma tumor models (B16F10 cells) by i.v. administration every 2 days, plus laser irradiation. Tumors in mice in the irradiated HMME-HA-SWCNTs arm showed the slowest growth (15% *vs.* saline controls).

A double polymer coating process involving SWNTs was recently proposed, in which chitosan was first adsorbed onto carboxyl SWNTs after which HA (MW 6 kDa) was grafted by amidation to the wrapping layer (Figure 7, B). Finally, a good quantity of DOX was loaded (48 % w/w) [265]. When administered to Sprague Dawley rats, there was a significant modification of blood parameters, although it was similar to that occurring with DOX. Pathological alterations were found in spleen

and lung tissues, indicating some response to the materials. These effects were more evident for the SWNT–chitosan. In a similar approach SWCNTs were considered for the delivery of salinomycin (SAL), a polyether antibiotic recently identified as a selective inhibitor of human breast cancer stem cells. In this system, SAL-SWCNT complexes were firstly prepared and then non-covalently wrapped with chitosan. HA was finally bonded to the outer chitosan layer. The resulting system was tested on $CD44^+$ subpopulations of human gastric cancer stem cells. Although the loading dose was around 40% (w/w) and the maximum release of SAL was 60%, HA-coated SWCNTs reduced growth of the tumosphere, inhibited the self-renewal capacity of CSCs, and enhanced apoptosis and necrosis, to a greater extent than did SAL-SWNTs-chitosan or free SAL, on this population [266].
Alternatively, PEI was also proposed to covalently coat MWCNTs. Improved activity of loaded DOX was observed on $CD44^+$ cell line [267].
It has also been proposed that SWCNTs be coated with HA, exploiting the hydrophobic properties of cholanic-derivatized HA (234 kDa) (see micelle section). Probe sonication was required to induce the hydrophobic interaction of cholanic acid with the SWCNT surface, and in turn disrupt the intertube van der Waals interactions. The HA chain was also chemically labeled with fluorescent dyes and a PET probe, to confirm selective CD44 receptor uptake in cells and *in vivo*. Biodistribution studies on an SCC7 tumor-bearing mouse model demonstrated rapid uptake by the RES organs, but long-term accumulation of HA-SWCNTs in the tumor regions [268]. An analogous approach, in which HA (750 kDa) was linked to phospholipids (DOPE, DLPE and DPPE), (Figure 7 , C), confirmed the increased solubility and reduced liver toxicity of SWCNT derivatives [269].
Interestingly, semiconducting SWCNT loaded with DOX have been compared with a HA-cholanic or phospholipid-PEG coating [270]; the nanoparticles were tested against MDR ovarian OVCAR8 cells. *In vitro* DOX nuclear localization demonstrated the superiority of HA-coated SWCNTs compared to untargeted particles. Mice xenografted with MDR OVCAR8 were given a single dose of targeted nanotubes (corresponding to 12 mg/kg DOX) and laser irradiation (808 nm) was applied after 24 h. Although biodistribution studies, by Raman spectroscopy, showed high liver and spleen localization, the targeted nanotubes combined with PTT afforded complete tumor eradication.

5.3.3 Graphene

Graphene oxide sheets (GO) possess a unique 2D structure (thickness 0.8-1.3 nm, lateral width 50-500 nm) and bear functional groups, including epoxy, hydroxyl, and carboxylic acid moieties, via which they can be conjugated with biomolecules [271]. Li *et al.* were the first to employ HA-conjugated GO in PDT studies. The GO was linked with HA (5.8 kDa), by reaction with ADH and conjugation with EDC (Figure 6, C). The nanomaterial afforded physical loading of the photosensitizer agent Ce6. When tested on HeLa cells, HA–GO/Ce6 demonstrated excellent solubility and improved photodynamic efficacy compared to free Ce6 [272]. Successively, HA (12 kDa) linked to GO through an EDA linker was used to deliver mitoxantrone (MIT), in order to mediate the photothermal effect and simultaneous drug release [273]. The biodistribution study showed prolonged circulation of HA-GO and prevalent excretion via the kidneys. Significantly enhanced antitumor efficacy for MIT/HA-GO plus laser irradiation, versus other groups, was reported although tumor growth was only monitored for six days. Other studies have reported that HA-GO can deliver DOX, without adding any photothermal effect [274].
Hybrid systems, such as HA/GO composite hydrogels, have been proposed to improve loading and release of small drugs. In particular, a larger amount of DOX can be loaded in HA-GO hydrogels than in HA hydrogels, thanks to the inclusion of GO in the gel network. Sustained release and prolonged anticancer activity were also reported [275].
Graphene quantum dots (GQDs) are nanometer-sized fragments of graphene have attracted great interest in the biomedical field, because of their performance in bioimaging: they possess stable and strong fluorescence, along with electrical and thermal conductivity. In comparison to colloidal QD, GQD do not possess intrinsic toxicity, and are highly stable to conjugation. To assess GQDs for

efficient receptor-mediated delivery as theranostic agent, HA-coated and DOX-loaded composite were proposed.
After anchoring HA through catechol linkage, the size of GQDs increased from 5–12 nm to 35–55 nm, but strong blue luminescence was observed in both cases. HA-GQDs can load ~75% of the DOX initially applied. On $CD44^+$ A549 cells, the native toxicity of GDQs was reduced after HA-derivatization. However, compared to DOX-loaded material, there was a significant increase in cytotoxicity. After i.v. administration in tumor bearing mice, both native GQDs and HA-modified GQDs were found in the liver and kidney, although the quantity of HA-GQD accumulating in tumor tissues was double that with native GDQs [276].

5.4 Mesoporous silica nanoparticles

Mesoporous silica nanoparticles (MSNPs) are now attracting attention as promising components of multimodal inorganic nanoparticle systems, owing to their straightforward synthesis, tunable pore size and shape, large loading capacity, good chemical stability, and good biocompatibility [277]. 'Smart' MSNPs can be further designed to be sensitive to internal stimuli (pH, GSH concentration, enzymes, or small molecules) or external stimuli (light, temperature, or magnetic field) [277]. In a recent development, MSNPs with pores of average diameter 2.9 nm were modified with 3-(2-aminoethylamino)propyltrimethoxysilane, after which HA (100 kDa) was conjugated by a carbodiimide reaction [278]. HA covered the MSNP pores, and complete release of rhodamine B, used as model drug, was achieved, but only after treatment with HYAL (Figure 6, D). Cytotoxicity assays of MSNP-HA loading DOX (at 68.5 μg/g SiO_2), using both CD44-expressing and control cell lines, showed a small but significant increase in activity on the $CD44^+$ cells.
A dual-stimulus responsive delivery system was achieved, by first loading DOX on MSNPs bearing thiol groups, then capping the surface through an amino-disulfide, and finally reacting the exposed amino groups with HA through EDC. Interestingly, a higher DOX DL (20% w/w) was reached using thiol MSNPs, whereas when NP-amino derivatives were used, due repulsive interactions reduced the loading dose to 3%. As expected, release was accelerated in the simultaneous presence of GSH and HYAL [279].
An improved technique has been described in which multifunctional silica mesoporous nanocapsules (MSNCs) decorated with HA demonstrated *in vivo* theranostic ability [280]. MSNCs with ultrasound-sensitive perfluorohexane and irinotecan encapsulated within, and a redox-responsive copolymer of PEG-disulfide linked to HA decorated on the outer surface, were produced. *In vitro* studies on targeting, followed by ultrasound imaging, demonstrated that binding to the CD44 receptor facilitates the accumulation of nanoparticles within cells. The ultrasound external pulsed stimulus afforded rapid release of the encapsulated drug. Nanoparticles with a hydrodynamic volume of 850 nm were then i.v. injected, and *in vivo* intensified ultrasound-guided high-intensity focused ultrasound (HIFU) ablation therapy was conducted on subcutaneously implanted HeLa tumor-bearing nude mice. The biodistribution study revealed elevated concentrations of nanoparticles in the lung, spleen, liver, and tumor. A synergic effect between HIFU ablation and HIFU-triggered release of irinotecan was achieved, with significant tumor regression.
Silica can also be employed to generate multi-structured solid core-shell nanoparticles (SNPs) with theranostic capacity. For example, SNPs with a fluorescent (FITC) core were produced using 3-(aminopropyl)-triethoxysilane, co-polymerized with tetraethylorthosilicate in an oil–water microemulsion. A coating of HA (31 kDa), covalently linked through an amide bond, increased the SNP's solubility, and produced CD44 targeting. In the latest step, DOX was linked to HA using conjugation with ADH, obtaining a DL of 0.6% (w/w) [281]. The activity and specificity were assayed on cell lines and in 3D cell models (SKOV-3 spheroids); it was found that the HA coating significantly enhanced the SNP's tumor penetration ability and activity, including against DOX-resistant ovarian cancer cells, compared to free drug [282].

A recent article describes a strategy whereby several properties can be collected into a single vehicle. Tubular shaped silica particles (SNTs) with a porous structure contained a compact SPION layer, loaded DOX, and could be coated with HA (SNT@SPION-DOX-HA) [283]. Briefly, the SNTs were fabricated by silica coating on a nickel-hydrazine complex, followed by acid etching. A deposit of SPION layer (average particle size 7 nm) was placed by thermodecomposition. The iron surface was then modified with a layer of dopamine, which enabled the subsequent adhesion of HA by electrostatic attraction. DOX was finally loaded into the hollow interior of SNT (pore size 0.8-2.5 nm) in a DL of 18.7% (w/w). Theranostic properties were evaluated by i.v. injection of SNT@SPION-DOX-HA into mice bearing 4T1 breast tumor on both flanks. MRI was first performed, applying a magnet only to the left flank. The magnetic field enhanced MR contrast (50% T2 signal intensity decrease) on the left flank, 24 h after administration. Biodistribution data revealed considerable accumulation in tumor tissue, although less marked than in the liver and spleen. On applying the magnetic field, there was a significant increase of nanocomposite localization in the tumor, together with a decrease in the RES organs.

6. Microparticles

Microparticles are solid particles in the size range 1-1000 μm, which that can be used as drug carriers; many different drugs may be incorporated into microparticles, and different materials may be adsorbed or chemically bonded onto them, for various purposes [284]. These carriers are used for a number of applications, including: to protect a drug, to achieve controlled release, to decrease toxic side effects, and to mask taste. Like nanoparticles, microparticles exist as microspheres, which are matrix systems in which the drug is dispersed within the polymer throughout the particle. At the same time they are also like microcapsules, which are vesicular systems in which the drug is located in an inner cavity surrounded by a continuous, thin polymer layer. The large number of materials and manufacturing methods available for microparticle production make them attractive and versatile tools for drug delivery.

Among the reported microcarriers, HA-associated microparticles are a heterogeneous family of structures, in which HA may be a constituent of the carrier (alone or associated to other agents), one of the components of the inner core, or even the polymeric material that modifies the microparticle surface. HA-microparticles have been studied extensively as long-lasting injectable soft tissue fillers, and as carriers in degenerative joint diseases, thanks to their ability to ensure sustained delivery and provide a high concentration of the active agent at the site of action [285, 286].

With regard to anticancer drug delivery, prolonged drug release is of especial importance, and thus HA-associated microparticles have been widely proposed as antitumor drug carriers. HA-microparticles are not only able to tune the release of the active agent, but can also promote targeted delivery of the antitumor drug. Moreover, the enhanced antitumor effect that these systems have shown, both *in vitro* and *in vivo,* compared to the free drug, has given HA-associated microparticles great potential for anticancer drug delivery, as many studies have reported.

HA was conjugated through its carboxylic groups to CDDP at a high temperature, to obtain "Hyplat" microparticles, in which HA was cross-linked to the drug. In these carriers, HA serves as a biodegradable and biocompatible polymeric matrix, onto which the drug may be linked, and at the same time as a targeting agent for the CD44 receptor. It was observed *in vitro* that OV2008 and A2780 $CD44^+$ cells internalized Hyplat more efficiently than did UCI101 $CD44^-$ cells. This microparticulate system had favorable pharmacokinetic characteristics since, after i.p. administration in mice, clearance of Hyplat from the peritoneum was reduced to one seventh, and tumor uptake was enhanced 2-3-fold in the $CD44^+$ tumor model compared to free drug. Moreover, Hyplat particles were more effective than the parent drug in inhibiting the growth of i.p. inoculated A2780 ovarian cancer cells [287].

MTX was encapsulated into a mixture of HA and sodium alginate (SA), with the goal of limiting its side effects, modulating its release, and protecting it from oxygen, pH, and other interactions. The microcapsules were prepared using the vibrational nozzle technique, by dropping a mixture of HA, alginate, and MTX into a calcium chloride solution; HA, alginate, and calcium chloride generated the encapsulating cross-linked barrier containing MTX; drug loading depended on the preparation conditions. HPLC analysis showed that the combination of SA and HA provided microcapsule stability. Encapsulated MTX showed controlled release and enhanced cytotoxicity in 5RP7 rat fibroblast cancer cell line [288].
A similar approach was adopted to prepare of radiosensitive liquid core microcapsules, composed of HA and SA, and encapsulating carboplatin. In the manufacturing procedure, a mixture of HA, SA, carboplatin, and a fluorescent marker was added to a calcium chloride solution supplemented with yttrium, to achieve calcium- and yttrium-induced polymerization. The addition of yttrium lead to microcapsules that more easily rupture. The antitumor effect was evaluated after microcarrier subcutaneous injection plus irradiation, on an inoculated Meth A fibrosarcoma in mice. The microcapsules exhibited a synergistic antitumor effect when combined with 2-Gy irradiation, showing increased intratumoral carboplatin concentration and prolonged drug release, which was a consequence of the decomposition of HA by radiation-induced superoxides [289].
For the purpose of gene delivery, HA was conjugated onto bPEI by the reductive amination reaction, to formulate plasmid DNA complexes encapsulated into PLGA microparticles. This approach aimed to diminish the cytotoxicity of bPEI and, at the same time, to achieve higher nucleic acid entrapment and more prolonged expression of DNA. The HA negative charges mitigate the positive charges associated with bPEI; moreover, the presence of HA oligosaccharides may increase DNA loading, since they stabilize the DNA/bPEI complexes. Lastly, the incorporation of an acid, HA, into the microparticulate system accelerated degradation of the PLGA, thus accelerating the release of DNA from the microparticles incorporating bPEI-HA, and enhancing cell transfection of the complexes [290].
HA was also used to modify the surface of microparticles, to obtain targeted drug delivery to tumor cells. In one approach, microbubbles (microparticles with a gas core) were prepared with poly(vinyl alcohol) (PVA), a polymer used in biomedicine as a bioinert support material [291]. In this system, cross-linked PVA chains formed the shell of microbubbles that contained an air-filled core, so that these carriers could be used as ultrasound contrast agents. To add HA to microbubbles, different aldehyde group-bearing HA derivatives were obtained by oxidation, and were then coupled onto the surface of PVA microbubbles by acetalization. DOX was also loaded into PVA-shelled microbubbles, deriving liquid-filled PVA microcapsules, with the goal of obtaining a new theranostic tool. Analysis of the *in vitro* behavior of these microcarriers on a $CD44^+$ HT-29 cell line showed that endocytosis was increased by the presence of the HA coating, which was characterized by a higher degree of oxidation. Moreover, drug release was prolonged when the HA-coating was present [292]. The same research group prepared injectable microgel particles of PVA coated with HA. Microgels are a class of three-dimensionally cross-linked hydrogels, confined within micrometer-sized particles. In this study, PVA derivatives (azide-bearing PVA and alkyne-bearing PVA, molar ratio 1:2) were cross-linked through click chemistry (azide-alkyne cycloaddition), and microgels were formed by combining the synthetic approach with the inverse emulsion droplet technique. The PVA-based microparticles were then coated with HA using the click chemistry approach, by reacting residual alkyne groups on the microgel surface with HA-azide. Click chemistry is a well-known synthetic strategy that presents several positive features, including high yields, mild reaction conditions, and the absence of toxic by-products [293]. *In vitro* tests showed that HA-coated microgels were internalized into HT-29 cells to a greater extent than uncoated microgel particles. DOX was then adsorbed onto the HA-coated microgel surface; drug adsorption was stabilized by the presence of the negative charges of HA, and the HA network also lead to sustained *in vitro* drug release [294].

7. Hydrogels

As well as the HA-based drug delivery systems described above, HA hydrogels formulation have found numerous biological applications in medicine and in cosmetic preparations, thanks to their high water content and consequent biocompatibility. Hydrogels have been successfully used as non-immunogenic filler, in cell delivery for tissue regeneration, as 3D cultures for cell expansion and recovery, in the study of tumor models, wound healing, and molecule delivery; the market is continuously increasing world-wide [295-298].

HA hydrogels are complex 3D cross-linked matrixes, that swell with water absorption and shrink on degradation, in which drugs can be loaded by either association or through covalent linkage [299]. HA hydrogels fall into the category of "living" HA derivatives, i.e. conjugates able to form further covalent bonds in the presence of other molecules or cells. The mechanical properties (stiffness, viscosity), drug content, and rate of biodegradation of HA hydrogels can be tuned by varying different parameters, such as HA MW, cross-linkages, and the percentage of derivatization. Injectable hydrogels are of considerable interest, presenting a number of advantages: they can take on the shape of the target cavity, and only require minimally-invasive surgery, thus reducing the risk of infection and complications. Nevertheless, despite their versatility, tenability, and the wide range of applications of these systems, some biological properties of hydrogels (such as non-toxicity and ability to incorporate molecules) must be taken into account if they are to be used for biomedical applications. The production of hydrogels must consider the biological compatibility of the different strategies of cross-linking chemistry, including the choice of reagents and by-products, which must be non-toxic and non-immunogenic. Moreover, appropriate molecule loading and gradual release must be achieved: for example, the incorporation of small drug molecules may limit applicability for the passive diffusion of such drugs out of highly porous hydrogels, or drug-polymer interactions may decrease drug release. Lastly, the rate of degradation is another crucial point: in some cases, multi-stimulus responsive degradation is required to assure complete drug release [299].

With regard to the chemical approach, HA hydrogels can be formed using addition and condensation reactions, usually through disulfide, hydrazide, enzymatic, and click reactions; additionally, HA hydrogels can be obtained by photopolymerization and electropolymerization, or by the combination of different techniques. For a complete review see Burdick J.A. *et al*. [299].

With regard to HA-based drug delivery, there is much interest in the field of anticancer therapy, since HA hydrogels can be employed to achieve both slow, controlled release of the drug, and enhanced antitumor activity. Sustained-release systems can increase local drug concentrations, and thus reduce systemic drug levels. Several of the above synthesis strategies have been applied to obtain HA cross-linked matrixes loaded with anticancer drugs. Moreover, some approaches have produced more complex structures, so as to further improve the efficacy of HA hydrogels, by preparing HA copolymers and hybrid matrixes.

Among anticancer drugs, CDDP was incorporated into an in-situ cross-linkable HA hydrogel, obtained from two polymers: HA-ADH and HA-aldehyde (200 and 10 kDa). *In-vitro* tests on the HA hydrogel showed that the speed of gelation and the period of sustained release of CDDP could be controlled by changing the concentration of the polymers. After i.p. administration in a mouse model of peritoneal carcinomatosis, the CDDP-HA hydrogel released the drug through matrix degradation mediated by peritoneal HYAL, and produced a stronger antitumor effect than the free drug [300]. CDDP was also loaded into a thermosensitive HA hydrogel, grafted first to poly(*N*-isopropylacrylamide) (PNIPAM) and then to gelatin, for intravesical administration in rats. The resulting hydrogel presented a highly fibrous structure, thanks to the addition of gelatin. This system can act as drug depot, owing to its bioadhesion to the bladder mucosa, enabling it to extend drug exposure in the bladder cavity beyond the voiding of urine. *In vivo,* the hydrogel doubled the

drug concentration in the bladder wall compared to a CDDP aqueous solution [301], probably because of the strong interactions between CDDP and the copolymers that reduce the delivery rate. Other research groups have developed anticancer drug-loaded thermoresponsive HA hydrogels, which undergo sol-gel phase transition under the stimulus of body temperature; in particular, hexamethylene diisocyanate and Pluronic F127 were added to HA to form an injectable DOX-loaded hydrogel, which self-assembled into a micellar structure at 37 °C, and maintained that structure for 30 days. The hydrogel degradation time could be extended and tuned by varying the concentration of hexamethylene diisocyanate and Pluronic F127. This system showed *in vitro* and *in vivo* sustained drug release [302]. A similar approach was reported by another research group [303].

A HA-based hydrogel was prepared to incorporate PTX in microparticulate form (diameter > 100 μm), then compared to a Taxol®-based hydrogel (PTX micelles of 14 nm). The microparticulate PTX hydrogel gradually released PTX *in vitro* when degraded by HYAL, while the Taxol®-based formulation showed low drug retention. After i.p. administration to tumor-bearing nude mice, the microparticulate HA-PTX gel lead to prolonged drug retention in the peritoneal cavity, but had a comparable anticancer effect to the Taxol®-based formulation. The study authors suggested these results were probably due to the limited dissolution of PTX in the small volume of the peritoneal region [304].

Four-arm PEG cross-linked HA hydrogels were prepared to load tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), a type II transmembrane protein, which in humans has 281 amino acids, and its PEGylated analogue (PEG-TRAIL). The negative charge of HA, due to the carboxylic acids, strongly binds to the positive charge of TRAIL. *In vitro,* PEG-TRAIL was more stable in HA hydrogels than was TRAIL (14 and 3 days, respectively); PEGylation probably decreased the initial burst release. The antitumor activity of loaded hydrogels was evaluated in MiaPaca-2 cell-xenografted mice. PEG-TRAIL release was faster *in vivo* than it was *in vitro*; however, antitumor activity was stronger than that of HA-associated TRAIL. This system may be considered safe, since it consists of only HA and PEG, which are both suitable for *in vivo* use in humans [305, 306] .

Tyramine was added to HA injectable hydrogels, through the oxidative coupling reaction of the tyramine moieties, catalyzed by hydrogen peroxide and horseradish peroxidase; a protein, interferon-α2a (IFN-α2a), was incorporated into these hydrogels, which are of varying stiffness, for liver cancer treatment. After subcutaneous injection in HAK-1B bearing mice, HA-tyramine hydrogels produced higher IFN-α2a concentrations in both plasma and tumor tissue (up to 3-fold) than did an IFN-α2a solution. Moreover, in tumor-bearing mice, loaded hydrogels effectively inhibited tumor growth and angiogenesis [307].

An example of a molecule covalently linked to a HA hydrogel for anticancer treatment is bovine serum amine oxidase; this molecule induces cytotoxicity through the oxidative stress caused by polyamine metabolites. To covalently immobilize the enzyme, cholesterol moieties were covalently linked to HA chains; carbodiimide chemistry was then used to link bovine serum amine oxidase lysine groups. The enzyme-loaded HA nanostructured injectable hydrogel showed increased anticancer activity on the M14 melanoma cell line than the free enzyme [308].

An acid-labile hydrazone linkage was formed to conjugate DOX to thiolated HA, so as to obtain dual-stimulus-response drug release. Once exposed to the air, an aqueous solution of the HA-DOX conjugate formed a thixotropic hydrogel. DOX could be released in pH- and reduction-responsive modes, under conditions mimicking the intracellular environment of cancer cells. In particular, the cumulative release of conjugated DOX was significantly accelerated at a mildly acidic pH of 5.0–6.0, and in the presence of GSH. The DOX-HA hydrogel was tested *in vitro* on human nasopharyngeal carcinoma CNE2 cells, and showed good anticancer activity [309].

Bisphosphonate was conjugated to cross-linked HMW-HA, and the activity of this injectable hydrogel was evaluated *in vitro,* on the more-$CD44^+$ HCT-116 cells, and the less-$CD44^+$ HEK-293T cells. Preliminary results showed that the specific drug release was triggered by degradation

of the HA-bisphosphonate conjugate mediated by HYAL, and that the smaller fragments of the HA-bisphosphonate prodrug were cell internalized by receptor-mediated endocytosis [310].
HA hydrogel scaffolds have also been studied to encapsulate DNA and siRNA for anticancer therapy. In one approach, DNA/PEI polyplexes were loaded into PEG, HA, and protein fibrin cross-linked hydrogels (all materials that are currently used in the clinic) through a process called "caged nanoparticle encapsulation" (CnE); this technique allowed the incorporation of stable, non-aggregated polyplexes, which displayed *in vivo* activity, as shown using a chorionic chick embryo assay. These systems avoid the aggregation and inactivation of the polyplexes that often occur inside hydrogel scaffolds, since the CnE technique utilizes neutral saccharides (sucrose) and polysaccharides (agarose), which protect the polyplexes from aggregation and inactivation, during lyophilization and hydrogel formation, respectively. The study authors hypothesize this protection came about thanks to an increase in the viscosity of the gel precursor solution, and a caging effect of the agarose polymers to the polyplexes [311, 312].

8. HA as an excipient for anticancer drugs

The unique tertiary structure of HMW-HA (825 kDa) has been exploited, in association with specific ionic formulation components, to entangle different anticancer drugs (irinotecan, DOX, 5FU, MTX) into the HA meshwork, in an approach known as HyACT® technology (Alchemia Ltd.) [313, 314]. From the regulatory standpoint, the main advantage of this formulation is the absence of chemical modification of either HA or the drug. Preclinical data demonstrated anticancer activity and an improved safety profile. Clinical trials have been carried out on 5FU-refractory patients with metastatic colorectal cancer. Phase I trials, on twelve patients, showed that HA-irinotecan was safe, well-tolerated, and that the therapeutic effectiveness of irinotecan was not compromised [314]. A phase II trial, involving 41 patients, confirmed the benefits of the HA formulation, in terms of progression-free survival and safety [315]. A phase II trial of FOLF (folinic acid, 5FU) and HA-irinotecan plus cetuximab, is currently recruiting (NCT02216487). Unfortunately, (October 2014) Alchemia Ltd. announced that its pivotal Phase III trial in the treatment of patients with metastatic colorectal cancer (NCT01290783) had not met its primary endpoint of statistically-significant improvement in progression-free survival [316].

Table 2. Selected HA-based drug delivery systems with the most advanced *in vivo* results available.

Type	HA constituents	Drug(s)	D.L. (E.E.)%	Administration route	Disease (site of growth)	Application	Tumor model	Ref
Micelles	HA-PHis/TPGS2k	doxorubicin	10	i.v.	Breast cancer (s.c.)	Imaging	MCF-7/ADR	[119]
Micelles	HA-ss-DOCA	paclitaxel	34	i.v.	Breast cancer (s.c.)	Imaging, antitumor effect, toxicity	MDA-MB-231	[120, 121]
Micelles	HA-ss-(OA-g-bPEI)	paclitaxel, AURKA siRNA	13 (PTX)	i.v.	Breast cancer (s.c.)	Imaging, antitumor effect	MDA-MB-231	[122]
Micelles	HA-5β-cholanic acid	paclitaxel	7.7	i.v.	Squamous cell carcinoma (s.c.)	Antitumor effect	SCC7	[127]
Micelles	FA-HA-C18	paclitaxel	15	i.v.	Breast cancer (s.c.)	Pharmacokinetics, biodistribution	MCF-7	[130, 131]
Micelles	HA/ATRA-PEI	ATRA	-	i.v.	Colon cancer (s.c.)	Antitumor effect/immunomodulator	CT-26, HCT-8	[135]
Micelles	MLDC	doxorubicin, ApoG2	13	i.v.	Prostatic cancer (s.c.)	Biodistribution, antitumor effect	PC-3	[136]
Liposomes	HA, phospholipids, cholesterol	mitomycin	(53)	i.v.	Different human models in mice	Toxicity, pharmacokinetics, biodistribution, antitumor effect	Different cancer cell lines	[161]
Liposomes	HA, phospholipids, cholesterol	doxorubicin	(78)	i.v.	Different human models in mice	Pharmacokinetics, biodistribution	Different cancer cell lines	[162]
Liposomes	HA-ceramide, phospholipids, cholesterol	doxorubicin - Magnevist	1.6	i.v.	Breast cancer (s.c.)	Imaging, pharmacokinetic	MDA-MB-231	[158]
Liposomes	HA-DPPE, phospholipids, cholesterol	Gemcitabine prodrug	(89)	i.p.	Pancreatic cancer (s.c.)	Antitumor effect	MiaPaca2	[154, 159]
Liposomes	HA, phospholipids, cholesterol	paclitaxel	(97)	i.v.	Hepatocellular cancer (s.c.)	Imaging, antitumor effect	HepG2	[163]

Liposomes	HA, phospholipids, cholesterol	miR34a beacon	-	i.v.	Breast cancer (s.c.)	Imaging	MDA-MB-231	[144]
Liposomes	HA, phospholipids, cholesterol	^{3}H-lipids, cyanine		i.v.	Breast cancer (s.c.)	Pharmacokinetics, biodistribution, imaging	MDA-MB-231	[146]
Polymeric nanoparticles	HA-PLGA	docetaxel	3	i.v.	Breast cancer (s.c.)	Pharmacokinetics, biodistribution, antitumor effect	MDA-MB-231	[176]
Polymeric nanoparticles	HA-PEG-PLGA	doxorubicin	(> 88)	i.v.	Ehrlich ascites tumor (s.c.)	Toxicity, biodistribution, antitumor effect	EAT	[177]
Polymeric nanoparticles	HA-PEG-PCL	doxorubicin	(> 85)	i.v.	Ehrlich ascites tumor (s.c.)	Toxicity, biodistribution, antitumor effect	EAT	[178]
Polymeric nanoparticles	HA-PEG-PLGA	5fluorouracil	(80)	i.v.	Ehrlich ascites tumor (i.p.	Toxicity, biodistribution, antitumor effect	EAT	[179]
Polymeric nanoparticles	HA-ss-PLGA	doxorubicin	8	i.v.	Breast cancer (s.c.)	Biodistribution	MDA-MB-231	[181]
Polymeric nanoparticles	HA-ss-PLGA	doxorubicin, cyclopamine	(71 DOX, 58 CYC)	i.p.	Breast cancer (s.c.)	Antitumor effect	MDA-MB-231	[182]
Polymeric nanoparticles	HA-PCL	doxorubicin	9	i.v.	Squamous cell carcinoma (s.c.)	Biodistribution, antitumor effect	SCC7	[183]
Polymeric nanoparticles	HA-PBCA	paclitaxel	(90)	i.v.	Sarcoma (s.c.)	Antitumor effect	S-180	[187]
Polymeric nanoparticles	HA-PBCA/TPGS	morin	7	i.v.	Sarcoma (s.c.)	Antitumor effect	S-180	[188]
Polymeric nanoparticles	HA-PDIPASP	doxorubicin, chlorin e6	14 (DOX)	i.v.	Colon cancer (s.c.)	Tumor accumulation, antitumor effect	CT-26	[189]
Polymeric nanoparticles	PLGA	folate- paclitaxel, HA-baicalein	(91 PTX, 88 baicalein)	i.v.	Lung cancer	Antitumor effect	A549	[190]
Lipid nanoparticles	HA-ceramide	docetaxel	8	i.v.	Breast cancer (s.c.)	Tumor targeting	MCF-7-ADR	[192]
Lipid nanoparticles	HA-ceramide	doxorubicin	6	i.t., i.v.	Melanoma (s.c.)	Antitumor effect	B16F10	[193]

Lipid nanoparticles	HA-ceramide-PEG	doxorubicin	12	i.v.	Squamous cell carcinoma (s.c.)	Tumor targeting, pharmacokinetics, antitumor effect	SCC7	[194]
Lipid nanoparticles	HA-5β-cholanic acid-PEG	doxorubicin, camptothecin	32 (DOX), 34 (CPT)	i.v.	Breast cancer (s.c.)	Antitumor effect	MDA-MB-231	[197]
Lipid nanoparticles	HA-5β-cholanic acid-PEG	Irinotecan	21	i.v.	Colon cancer (s.c.)	Antitumor effect	HT-29	[199]
Lipid nanoparticles	HA-5β-cholanic acid-PEG-APMA	paclitaxel	6	i.v.	Squamous cell carcinoma (s.c.)	Biodistribution, antitumor effect	SCC7	[201]
Lipid nanoparticles	HA-5β-cholanic acid-PEG/CaP	doxorubicin	8	i.v.	Squamous cell carcinoma (s.c.)	Biodistribution, antitumor effect	SCC7	[202]
Lipid nanoparticles	HA-lipoic acid	doxorubicin	12	i.v.	Breast cancer (s.c.)	Biodistribution, antitumor effect	MCF-7-ADR	[203]
Lipid nanoparticles	HA-all-trans retinoic acid	paclitaxel	29	i.v.	Melanoma (s.c.)	Antitumor effect	B16F10	[204]
Lipid nanoparticles	HA-tocopheryl succinate/TPGS	docetaxel	6	i.v.	Breast cancer (s.c.)	Biodistribution, antitumor effect	MCF-7/ADR	[206]
Lipid nanoparticles	HA-glycyrrhetinic acid	doxorubicin	34	i.v.	Hepatocellular carcinoma (s.c.)	Biodistribution, antitumor effect	HepG2	[208]
Lipid nanoparticles	HA-aminooctane, HA-PEG	cisplatin	18-20	i.v.	Lung cancer (s.c.)	Biodistribution	A549	[210]
Lipid nanoparticles	HA-CHEMS	docetaxel	3	i.v.	Breast cancer (s.c.)	Biodistribution, antitumor effect	MCF-7	[211]
Lipid nanoparticles	HA-CHEMS	docetaxel	10	i.v.	Breast cancer	Pharmacokinetics, biodistribution, antitumor effect	4T1	[212]
Solid lipid nanoparticles	HA/lipids	paclitaxel	4	i.v.	Melanoma i.v.	Biodistribution, antitumor effect	B16F10	[214]
Nanostructured lipid carriers	HA-alendronate, lipids	Irinotecan	-	i.v.	Ehrlich ascites tumor (s.c.)	Biodistribution, antitumor effect	EAT	[219]
Nanostructured lipid carriers	HA/lipids	5fluorouracil-stearic acid + cisplatin	(90 5FU-stearic acid, 89	i.v.	Gastric cancer	Antitumor effect	BGC823	[220]

			CDDP)					
Nanostructured lipid carriers	HA/lipids	paclitaxel	4	i.v.	Melanoma (s.c.)	Pharmacokinetics, biodistribution, antitumor effect	B16	[221]
GAGs	**HA, phospholipids**	**paclitaxel**	**(100)**	**i.v.**	**Colon cancer (s.c.)**	**Biodistribution, pharmacokinetics, antitumor effect**	**CT-26**	**[222]**
GAGs	**HA, phospholipids**	**doxorubicin**	**(60)**	**i.v.**	**Ovarian cancer (s.c.)**	**Biodistribution, pharmacokinetics, antitumor effect**	**NAR-GFP**	**[225]**
GAGs	**HA, phospholipids, cholesterol**	**methotrexate**	**(>60)**	**i.v.**	**Melanoma (s.c.)**	**Biodistribution, antitumor effect**	**B16F10**	**[226]**
Coated nanogels	HA-chitosan + Eudragit S100	Oxaliplatin	(40)	Os	Colon cancer	Biodistribution, antitumor effect	HT-29	[230]
Nanogels	HA/chitosan	doxorubicin + MiR-34a	(48 DOX, 91 MiR-34a)	i.v.	Breast cancer (s.c.)	Antitumor effect	MDA-MB-231	[231]
Complexes	HA-PEI/HA-PEG	MDR1 siRNA	-	i.v.	Ovarian (s.c.)	Antitumor effect	OVCAR8TR	[233]
Complexes	HA-PEI/HA-PEG	MDR1 siRNA	-	i.v.	Ovarian (s.c.)	Antitumor effect	SKOV-3TR	[234]
Complexes	HA-g-PEG, PCL-g-PDMAEMA	pDNA	-	i.v.	Hepatocellular carcinoma	Gene expression	HepG2	[235]
Complexes	DNA, methacrylated HA and protamine	doxorubicin	-	i.v.	Breast cancer (s.c.)	Biodistribution, antitumor effect	MDA-MB-231	[236]
HA-nanogels	Glutaraldheyde-crosslinked HA	paclitaxel	1- 8	i.t.	DMBA-induced mammary tumor	Antitumor effect	DMBA-induced mammary tumor	[237]
HA-nanogels	Methacrylated HA	doxorubicin	16	i.v.	Hepatoma (s.c.)	Biodistribution, antitumor effect	H22	[238]
HA-nanogels	HA-cholesteryl-drug	curcumin	-	i.v., i.p., os	Pancreatic (s.c.), mammary	Biodistribution, pharmacokinetics,	MiaPaCa2, 4T1	[240]

						antitumor effect		
AuNPs	Oligo-HA-thiol	-		i.t., iv	Ovarian cancer, (s.c)	Imaging	OVCAR-3	[249]
AuNPs	Thiol-HA-drug	pheophorbide A		Iv	Lung cancer, (s.c.)	Imaging+ PTT+ PDT	A549	[250]
Fe_3O_4 NPs	PEI-F-HA	fluorescein		Iv	Cervix adenocarcinoma (s.c)	Imaging (MRI), biodistribution	U87MG, HeLa	[252]
C_{60}	HA-C_{60}	-		i.v.	Colon cancer (s.c.)	Imaging+ PDT	HCT-116, KB	[257]
MWCNTs	HA-PEG-CNT	doxorubicin	26-32%	i.v.	SD rats	Antitumor effect, biodistribution, toxicity	EAT	[263]
SWCNTs	HA-CNT	Hemoporfin	70%	i.v.	Melanoma (s.c.)	Antitumor effect (PDT, PTT)	B16F10	[264]
SWCNTs	Chitosan + HA	doxorubicin	48%	i.v.	Toxicity on SD rats	Toxicity	-	[265]
SWCNTs	Cholanic	Fluorescent+PET probes		i.v.	Squamous cell carcinoma (s.c.)	Biodistribution	SCC7	[268]
GO	HA-GO	mitoxantrone	45%	i.v.	Breast cancer (s.c.)	Imaging+ PTT, pharmaco-kinetics on SD.	MCF-7	[273]
GQD	HA-dopamine	doxorubicin	-	i.v.	Lung cancer	Imaging, biodistribution	A549	[276]
MSNP	PEG-c-s-HA	Irinotecan + PFH	34% (drug)	i.v.	Hepatocellular, ovarian cancer	Imging+ HIFU	HepG2, HeLa	[280]
SNT@SPION		doxorubicin	18.7%	i.v.	Breast cancer (s.c.)	Imaging, biodistribution	4T1	[283]
Microparticles	Crosslinked HA-cisplatin (Hyplat)	cisplatin	27	i.p.	Ovarian carcinoma (i.p.)	MTD, pharmacokinetics, biodistribution, survival	A2780	[287]
Microparticles	HA-alginate	carboplatin	-	s.c.	Fibrosarcoma (s.c.)	Antitumor effect	Meth A	[289]
Hydrogel	HA-ADH/HA-aldehyde	cisplatin	-	i.p.	Gastric cancer (i.p.)	Antitumor effect	MKN45P	[300]
Hydrogel	HA-PNIPAM-gelatin	cisplatin	-	intravesical	-	Drug permeation, safety	-	[301]
Hydrogel	HA/HDI-PF127	doxorubicin	-	i.t.	Breast cancer (s.c.)	Antitumor effect	MCF-7	[302]
Hydrogel	HA-ADH/HA-aldehyde	paclitaxel	-	i.p.	Ovarian cancer (i.p.)	Antitumor effect	SKOV-3	[304]
Hydrogel	HA-PEG	TRAIL, PEG-	-	i.t.	Pancreatic cancer	Antitumor effect	Mia Paca-2	[306]

		TRAIL			(s.c.)			
Hydrogel	HA-tyramine	IFN-α2a	-	s.c.	Hepatic cancer (s.c.)	Pharmacokinetics, antitumor effect	HAK-1B	[307]
Proprietary Formulation	HyACT	Irinotecan, doxorubicin, 5-fluorouracil	-	i.v.	Refractory Metastatic Colorectal Cancer Patients	Phase II	-	[314, 315, 317]

i.p., intraperitoneal administration or implant; i.t., intratumoral; i.v., intravenous; s.c., subcutaneous; SPI, polymeric micellar immunomodulator; ATRA, all-trans-retinoic acid; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; EAT, Ehrlich ascites tumor; SD, breast-tumor-induced Sprague-Dawley female rats; PFH, perfluorohexane; HIFU, high-intensity focused ultrasound; MTD, maximum tolerated dose; PCL-PDMAEMA, (polycaprolactone-graft-poly(*N*,*N*-dimethylaminoethyl methacrylate); PDIPASP, poly((2-diisopropylaminoethyl)aspartimide), PBCA, poly(butyl cyanoacrylate); ApoG2, apogossypolone.

9. Conclusions and perspectives

Hyaluronic acid is a very attractive molecule for use as a vehicle, which can overcome the limitations upon the administration of conventional anticancer drugs. It has also been used as a targeting moiety, as it can reach the CD44 receptor, which is overexpressed in many tumors and on CSCs. In terms of clinical trials, the most advanced technologies are conjugates and injectable formulations: as the literature shows, in both cases, products have reached phase II or III clinical trials.

The formulation of conjugates has both advantages and limitations. Starting with preparation, this approach is feasible to obtain a clearly-characterized drug product with a mean value of derivatization, and little variability between batches. From the pharmaceutical standpoint, these features allow the process to be scaled up. The solubility profile of highly hydrophobic drugs is improved, and the native drug can be released after degradation of the linkage between drug and polymer. In most cases this reduces drug toxicity permits to increase the dosage, providing sustained release with clear anticancer benefits. Finally, by using HAs of different MWs, binding to CD44 is maintained, at least judging from what has been demonstrated *in vitro*. Certainly, in the case of parenteral routes, further studies will be needed to limit off-targeting to healthy tissue, in particular to the liver, which is the principal elimination organ of HA.

From the regulatory standpoint, HA-conjugates are new chemical entities, thus physico-chemical characterization, starting with the drug release profile, is mandatory. To circumvent some regulatory problems, the interesting approach proposed by Alchemia Ltd, i.e. the non-covalent interaction formulation composed of HMW HA and drugs, appears more feasible. Clinical results demonstrated a marked reduction of side effects, but efficacy is apparently not improved over that of standard protocols. This is one of the major challenges for all novel delivery systems compared with well-consolidated protocols: the majority of HA-conjugates, and also the dispersed systems reviewed here, employed the most conventional cytotoxic drugs as payload.

In the light of the preclinical evaluation of Bivatuzumab-mertansine (a monoclonal antibody directed to CD44, linked to a very toxic payload) [318] one solution might be to improve the activity of the HA-conjugate, especially to overcome tumor MDR, by linking more toxic/potent agents. Appropriate linkers to fine-tune drug release will be necessary to improve the therapeutic window. Furthermore, for all the reasons mentioned above, it currently appears likely that HA-conjugates, like hydrogels, will be reserved for intracavitary or locoregional administration.

Regarding dispersed systems, from micelles to microparticles, a large body of research produced by many groups in the last few years demonstrates the vitality of this field; these studies have explored different approaches to find candidates for further clinical evaluation.

Naturally, each of the systems offers advantages, confirmed by promising *in vivo* results, but also raises many questions, such as the effect of particle size on biodistribution, their strong or weak stability, or drug burst release, which likely impacts on pharmacokinetics, tissues distribution, and drug efficacy. However, the goal of the review was to discuss how HA could improve pharmacological effects. It is believed that, like PEG, HA can form a corona surrounding the particles, which can repulse opsonins and, as a consequence, increase nanoparticles' blood half-life. Few studies have investigated the effect of HA on particle pharmacokinetics. It appears that nanosystem blood circulation is critically important for active tumor targeting [146]. Studies in this field show HA to be much less efficient than PEG to increase circulation time, but fail to demonstrate any complement activation effect, as reported for PEG. Further, HA affects tumor targeting, but although in many cases this effect is clearly shown in *in vitro* models, *in vivo* there is little evidence that tumor targeting is closely related to the CD44 targeting effect. More work must be done in this field to properly evaluate the advantages of the HA corona in terms of tumor targeting.

Combining the high intracellular internalization, due to HMW HA's multivalent binding to receptors, with an appropriate degree of PEGylation to provide long circulation and HA charge shielding, while carefully balancing the two, will improve the *in vivo* characteristics of all nanosystems [319] (Figure 8).

By exploiting both the EPR effect and HA targeting capacities, interesting tumor localization concentrations have been achieved (i.e. the percentage of injected dose/mass tissue). The organ and tumor distribution of nanosystems can readily be determined using NIR dyes, although the concentration of the drug in tumor/organs is the true measure of the system's potency: achieving locally-elevated drug concentrations is a requirement to improve efficacy over that of standard drug solution and, by saturating the cellular drug efflux pumps, it may overcome MDR. Preclinical trials that quantify both pharmacokinetics and tumor accumulation will be important to clearly assess the effect of EPR on the uptake of a drug into solid tumors [320].
However, an important point is that most studies only compare the effectiveness of drug-loaded nanosystems with their respective free drugs, whereas very few attempts have been made to make head-on comparisons with clinically-available nanoparticulate formulation counterparts (such as Caelyx®, Myocet®, Abraxane®). These systems provide the best balance currently available between drug loading, *in vitro* binding, *in vivo* stability, and clinical safety. These are all points to be considered in depth at the development stage of a novel nanosystem.
From the results reviewed here, a picture has emerged that organic nanosystems composed of HA may function as promising anticancer tools. However, especially for the most promising systems, validated studies on toxicity, biodistribution, dosing schedules, and dose intensities must be awaited, as has occurred with Caelyx®, and HA-PTX [83, 321], before the true clinical advantages clearly emerge.
Concerning gene therapy HA is a useful tool to reduce the positive charge density of nucleic acid-polycation polymer complexes. However, although they give nucleic acid vectors improved stability and decreased toxicity, the complexity of these systems is further increased.
Lastly, inorganic particles are attracting rapidly-increasing attention. These systems can offer outstanding DL values (e.g. for DOX), combining therapeutic, diagnostic, and trigger-release properties in a single agent. It is clear that magnetic, light, heat, and other external stimuli can increase the specificity to the target, and give rapid drug release. Particular promise is shown by CNTs, in which the photothermal effect can significantly enhance the anticancer effect [270]. However, the combination of different components in a single system increases its complexity, and detracts from industrial feasibility. Furthermore, it is by no means obvious that these systems can overcome difficulties concerning toxicity, and meet regulatory standards, in order to be applied in the clinic.
In conclusion, the examples reported in the present review indicate that many interesting approaches involving HA are now evolving. Although additional research will be required before they can reach patients' beds, the promising results obtained thus far fully justify further multidisciplinary efforts, in terms of increased investment and further investigations in this field.

10. Acknowledgements

The work was partially funded by MIUR-University of Torino "Fondi Ricerca Locale (ex-60%)" and Galileo project 2014-2015 Università Italo-Francese.

Figures captions

Figure 1. Structure of hyaluronic acid and scheme of main reactions producing derivatives.

Figure 2. Publications over the last 25 years, and citations of the topic 'Hyaluronic acid AND drug delivery'. Source: Elsevier's Scopus®.

Figure 3: Structures of the principal drug derivatives described in the text. R'= A) Butyrate. R= B) paclitaxel linked in 2'-position by a succinic ester to ADH linker; C) ONCOFID-S; D) doxorubicin linked at 13-carbonyl through an ADH linker; E) cis,cis,trans-dichloro-hydroxyl-succinato-platinum(IV) linked with ethylenediamine spacer.

Figure 4. HA derivatives involved in micelle preparation. Anticancer drugs or siRNA can be loaded and delivered in micelles made by HA linked to different hydrophobic moieties.

Figure 5. Principal HA derivatives involved in nanoparticle preparation.

Figure 6. Inorganic nanocomposites with HA. A) Graphene oxide sheet linked to HA through ADH linker; B) C_{60}-HA derivatives; C) HA-bearing doxorubicin linked to SPION; D) Mesoporous silica nanoparticle coated with HA; release induced by HYAL is depicted.

Figure 7. Examples of different approaches to conjugate HA to carbon nanotubes. A) linkage using a hydrophilic spacer (doxorubicin as loaded drug); B) a double layer with non-covalent adsorption of chitosan and then HA (salincomycin as loaded drug); C) phospholipids as linking arm.

Figure 8. Illustration of the *in vivo* biodistribution of HA-based nanosystems. The key to achieving optimal antitumor efficacy is to find a favorable balance between long circulation and targeted cellular uptake exploiting the EPR effect. A high local drug concentration can overcome cell MDR.

11. References

[1] R.K. Reed, K. Lilja, T.C. Laurent, Hyaluronan in the rat with special reference to the skin, Acta Physiol. Scand., 134 (1988) 405-411.

[2] L. Schaefer, R.M. Schaefer, Proteoglycans: from structural compounds to signaling molecules, Cell Tissue Res., 339 (2010) 237-246.

[3] B.P. Toole, Hyaluronan in morphogenesis, Semin. Cell Dev. Biol., 12 (2001) 79-87.

[4] R. Stern, A.A. Asari, K.N. Sugahara, Hyaluronan fragments: An information-rich system, Eur. J. Cell Biol., 85 (2006) 699-715.

[5] D. Jiang, J. Liang, P.W. Noble, Hyaluronan in tissue injury and repair, Annu. Rev. Cell Dev. Biol., 2007, pp. 435-461.

[6] M.G. Neuman, R.M. Nanau, L. Oruna-Sanchez, G. Coto, Hyaluronic Acid and Wound Healing, Journal of Pharmacy and Pharmaceutical Sciences, 18 (2015) 53-60.

[7] R.K. Sironen, M. Tammi, R. Tammi, P.K. Auvinen, M. Anttila, V.M. Kosma, Hyaluronan in human malignancies, Exp. Cell Res., 317 (2011) 383-391.

[8] E.A. Turley, P.W. Noble, L.Y.W. Bourguignon, Signaling properties of hyaluronan receptors, J. Biol. Chem., 277 (2002) 4589-4592.

[9] N. Volpi, J. Schiller, R. Stern, L. Šoltés, Role, metabolism, chemical modifications and applications of hyaluronan, Curr. Med. Chem., 16 (2009) 1718-1745.

[10] R. Stern, Association between cancer and "acid mucopolysaccharides": An old concept comes of age, finally, Semin. Cancer Biol., 18 (2008) 238-243.

[11] N. Itano, T. Sawai, M. Yoshida, P. Lenas, Y. Yamada, M. Imagawa, T. Shinomura, M. Hamaguchi, Y. Yoshida, Y. Ohnuki, S. Miyauchi, A.P. Spicer, J.A. McDonald, K. Kimata, Three isoforms of mammalian hyaluronan synthases have distinct enzymatic properties, J. Biol. Chem., 274 (1999) 25085-25092.

[12] D. Vigetti, M. Viola, E. Karousou, G. De Luca, A. Passi, Metabolic control of hyaluronan synthases, Matrix Biol., 35 (2014) 8-13.

[13] P.H. Weigel, V.C. Hascall, M. Tammi, Hyaluronan synthases, J. Biol. Chem., 272 (1997) 13997-14000.

[14] J.R.E. Fraser, L.E. Appelgren, T.C. Laurent, Tissue uptake of circulating hyaluronic acid - A whole body autoradiographic study, Cell Tissue Res., 233 (1983) 285-293.

[15] T.C. Laurent, The chemistry, biology and medical applications of hyaluronan and its derivatives, Portland Press, London1998.

[16] T.C. Laurent, J.R.E. Fraser, Hyaluronan, FASEB J., 6 (1992) 2397-2404.

[17] C. Underhill, CD44: The hyaluronan receptor, J. Cell Sci., 103 (1992) 293-298.

[18] R.J.S. Sneath, D.C. Mangham, The normal structure and function of CD44 and its role in neoplasia, Journal of Clinical Pathology - Molecular Pathology, 51 (1998) 191-200.

[19] P.N. Goodfellow, G. Banting, M.V. Wiles, A. Tunnacliffe, M. Parkar, E. Solomon, R. Dalchau, J.W. Fabre, The gene, MIC4, which controls expression of the antigen defined by monoclonal antibody F10.44.2, is on human chromosome 11, Eur. J. Immunol., 12 (1982) 659-663.

[20] V. Orian-Rousseau, CD44, a therapeutic target for metastasising tumours, Eur. J. Cancer, 46 (2010) 1271-1277.

[21] D. Naor, S. Nedvetzki, I. Golan, L. Melnik, Y. Faitelson, CD44 in cancer, Crit. Rev. Clin. Lab. Sci., 39 (2002) 527-579.

[22] J. Cichy, E. Puré, The liberation of CD44, J. Cell Biol., 161 (2003) 839-843.

[23] D. Naor, S.B. Wallach-Dayan, M.A. Zahalka, R.V. Sionov, Involvement of CD44, a molecule with a thousand faces, in cancer dissemination, Semin. Cancer Biol., 18 (2008) 260-267.

[24] S. Misra, P. Heldin, V.C. Hascall, N.K. Karamanos, S.S. Skandalis, R.R. Markwald, S. Ghatak, Hyaluronan–CD44 interactions as potential targets for cancer therapy, FEBS J., 278 (2011) 1429-1443.

[25] U. Günthert, M. Hofmann, W. Rudy, S. Reber, M. Zöller, I. Haußmann, S. Matzku, A. Wenzel, H. Ponta, P. Herrlich, A new variant of glycoprotein CD44 confers metastatic potential to rat carcinoma cells, Cell, 65 (1991) 13-24.
[26] M. Zöller, CD44: can a cancer-initiating cell profit from an abundantly expressed molecule?, Nat. Rev. Cancer, 11 (2011) 254-267.
[27] A. Jaggupilli, E. Elkord, Significance of CD44 and CD24 as cancer stem cell markers: An enduring ambiguity, Clin. Dev. Immunol., 2012 (2012).
[28] C.A. Maxwell, J. McCarthy, E. Turley, Cell-surface and mitotic-spindle RHAMM: Moonlighting or dual oncogenic functions?, J. Cell Sci., 121 (2008) 925-932.
[29] B. Yang, B.L. Yang, R.C. Savani, E.A. Turley, Identification of a common hyaluronan binding motif in the hyaluronan binding proteins RHAMM, CD44 and link protein, EMBO J., 13 (1994) 286-296.
[30] P.G. Telmer, C. Tolg, J.B. McCarthy, E.A. Turley, How does a protein with dual mitotic spindle and extracellular matrix receptor functions affect tumor susceptibility and progression?, Communicative and Integrative Biology, 4 (2011) 182-185.
[31] K.M. Gust, M.D. Hofer, S.R. Perner, R. Kim, A.M. Chinnaiyan, S. Varambally, P. Moller, L. Rinnab, M.A. Rubin, J. Greiner, M. Schmitt, R. Kuefer, M. Ringhoffer, RHAMM (CD168) is overexpressed at the protein level and may constitute an immunogenic antigen in advanced prostate cancer disease, Neoplasia, 11 (2009) 956-963.
[32] H. Shigeishi, S. Fujimoto, M. Hiraoka, S. Ono, M. Taki, K. Ohta, K. Higashikawa, N. Kamata, Overexpression of the receptor for hyaluronan-mediated motility, correlates with expression of microtubule-associated protein in human oral squamous cell carcinomas, Int. J. Oncol., 34 (2009) 1565-1571.
[33] D. Jiang, J. Liang, P.W. Noble, Hyaluronan as an immune regulator in human diseases, Physiol. Rev., 91 (2011) 221-264.
[34] N. Itano, K. Kimata, Altered hyaluronan biosynthesis in cancer progression, Semin. Cancer Biol., 18 (2008) 268-274.
[35] B.P. Toole, Hyaluronan: from extracellular glue to pericellular cue, Nat. Rev. Cancer, 4 (2004) 528-539.
[36] M. Wu, M. Cao, Y. He, Y. Liu, C. Yang, Y. Du, W. Wang, F. Gao, A novel role of low molecular weight hyaluronan in breast cancer metastasis, FASEB J., 29 (2015) 1290-1298.
[37] D.C. West, I.N. Hampson, F. Arnold, S. Kumar, Angiogenesis induced by degradation products of hyaluronic acid, Science, 228 (1985) 1324-1336.
[38] S. Misra, S. Ghatak, B.P. Toole, Regulation of MDR1 Expression and Drug Resistance by a Positive Feedback Loop Involving Hyaluronan, Phosphoinositide 3-Kinase, and ErbB2, J. Biol. Chem., 280 (2005) 20310-20315.
[39] D. Liu, E. Pearlman, E. Diaconu, K. Guo, H. Mori, T. Haqqi, S. Markowitz, J. Willson, M.S. Sy, Expression of hyaluronidase by tumor cells induces angiogenesis in vivo, Proceedings of the National Academy of Sciences, 93 (1996) 7832-7837.
[40] D. Jiang, J. Liang, J. Fan, S. Yu, S. Chen, Y. Luo, G.D. Prestwich, M.M. Mascarenhas, H.G. Garg, D.A. Quinn, R.J. Homer, D.R. Goldstein, R. Bucala, P.J. Lee, R. Medzhitov, P.W. Noble, Regulation of lung injury and repair by Toll-like receptors and hyaluronan, Nat. Med., 11 (2005) 1173-1179.
[41] K. Hosono, Y. Nishida, W. Knudson, C.B. Knudson, T. Naruse, Y. Suzuki, N. Ishiguro, Hyaluronan oligosaccharides inhibit tumorigenicity of osteosarcoma cell lines MG-63 and LM-8 in vitro and in vivo via perturbation of hyaluronan-rich pericellular matrix of the cells, Am. J. Pathol., 171 (2007) 274-286.
[42] S.S. Skandalis, C. Gialeli, A.D. Theocharis, N.K. Karamanos, Chapter Eleven - Advances and Advantages of Nanomedicine in the Pharmacological Targeting of Hyaluronan-CD44 Interactions and Signaling in Cancer, in: A.S. Melanie, H. Paraskevi (Eds.) Adv. Cancer Res., Academic Press2014, pp. 277-317.

[43] L. Liu, Y. Liu, J. Li, G. Du, J. Chen, Microbial production of hyaluronic acid: current state, challenges, and perspectives, Microbial Cell Factories, 10 (2011) 99.
[44] L. Liu, G. Du, J. Chen, Y. Zhu, M. Wang, J. Sun, Microbial production of low molecular weight hyaluronic acid by adding hydrogen peroxide and ascorbate in batch culture of Streptococcus zooepidemicus, Bioresour. Technol., 100 (2009) 362-367.
[45] Y. Wu, Preparation of low-molecular-weight hyaluronic acid by ozone treatment, Carbohydr. Polym., 89 (2012) 709-712.
[46] R. Stern, G. Kogan, M.J. Jedrzejas, L. Šoltés, The many ways to cleave hyaluronan, Biotechnol. Adv., 25 (2007) 537-557.
[47] X. Han, Z. Li, J. Sun, C. Luo, L. Li, Y. Liu, Y. Du, S. Qiu, X. Ai, C. Wu, H. Lian, Z. He, Stealth CD44-targeted hyaluronic acid supramolecular nanoassemblies for doxorubicin delivery: Probing the effect of uncovalent pegylation degree on cellular uptake and blood long circulation, J. Controlled Release, 197 (2015) 29-40.
[48] J. Fang, H. Nakamura, H. Maeda, The EPR effect: Unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect, Adv. Drug Del. Rev., 63 (2011) 136-151.
[49] X. Wei, T.H. Senanayake, G. Warren, S.V. Vinogradov, Hyaluronic Acid-Based Nanogel-Drug Conjugates with Enhanced Anticancer Activity Designed for the Targeting of CD44-Positive and Drug-Resistant Tumors, Bioconjugate Chem., 24 (2013) 658-668.
[50] M.K. Hertweck, F. Erdfelder, K.A. Kreuzer, CD44 in hematological neoplasias, Ann. Hematol., 90 (2011) 493-508.
[51] R.I.C. Russo, M.G. García, L. Alaniz, G. Blanco, E. Alvarez, S.E. Hajos, Hyaluronan oligosaccharides sensitize lymphoma resistant cell lines to vincristine by modulating P-glycoprotein activity and PI3K/Akt pathway, Int. J. Cancer, 122 (2008) 1012-1018.
[52] X. Xu, A.K. Jha, D.A. Harrington, M.C. Farach-Carson, X. Jia, Hyaluronic acid-based hydrogels: From a natural polysaccharide to complex networks, Soft Matter, 8 (2012) 3280-3294.
[53] H.G. Garg, C.A. Hales, Chemistry and biology of hyaluronan, Elsevier2004.
[54] C.E. Schanté, G. Zuber, C. Herlin, T.F. Vandamme, Chemical modifications of hyaluronic acid for the synthesis of derivatives for a broad range of biomedical applications, Carbohydr. Polym., 85 (2011) 469-489.
[55] M.N. Collins, C. Birkinshaw, Hyaluronic acid based scaffolds for tissue engineering—A review, Carbohydr. Polym., 92 (2013) 1262-1279.
[56] V.M. Plattt, F.C. Szoka Jr, Anticancer therapeutics: Targeting macromolecules and nanocarriers to hyaluronan or CD44, a hyaluronan receptor, Mol. Pharm., 5 (2008) 474 - 486.
[57] E.J. Oh, K. Park, K.S. Kim, J. Kim, J.-A. Yang, J.-H. Kong, M.Y. Lee, A.S. Hoffman, S.K. Hahn, Target specific and long-acting delivery of protein, peptide, and nucleotide therapeutics using hyaluronic acid derivatives, J. Controlled Release, 141 (2010) 2-12.
[58] T. Pouyani, G.D. Prestwich, Functionalized derivatives of hyaluronic acid oligosaccharides: Drug carriers and novel biomaterials, Bioconjug. Chem., 5 (1994) 339-347.
[59] P. Bulpitt, D. Aeschlimann, New strategy for chemical modification of hyaluronic acid: Preparation of functionalized derivatives and their use in the formation of novel biocompatible hydrogels, J. Biomed. Mater. Res., 47 (1999) 152-169.
[60] D. Coradini, C. Pellizzaro, G. Abolafio, M. Bosco, I. Scarlata, S. Cantoni, L. Stucchi, S. Zorzet, C. Turrin, G. Sava, A. Perbellini, M. Daidone, Hyaluronic-acid butyric esters as promising antineoplastic agents in human lung carcinoma: A preclinical study, Invest. New Drugs, 22 (2004) 207-217.
[61] L. Pravata, C. Braud, M. Boustta, A. El Ghzaoui, K. Tømmeraas, F. Guillaumie, K. Schwach-Abdellaoui, M. Vert, New amphiphilic lactic acid oligomer - Hyaluronan conjugates: Synthesis and physicochemical characterization, Biomacromolecules, 9 (2008) 340-348.
[62] H. Lee, K. Lee, T.G. Park, Hyaluronic Acid-Paclitaxel Conjugate Micelles: Synthesis, Characterization, and Antitumor Activity, Bioconjugate Chem., 19 (2008) 1319-1325.

[63] H. Ringsdorf, Structure and properties of pharmacologically active polymers, Journal of Polymer Science: Polymer Symposia, 51 (1975) 135-153.
[64] R. Duncan, R. Gaspar, Nanomedicine(s) under the microscope, Mol. Pharm., 8 (2011) 2101-2141.
[65] F.D. Battistini, M.E. Olivera, R.H. Manzo, Equilibrium and release properties of hyaluronic acid–drug complexes, Eur. J. Pharm. Sci., 49 (2013) 588-594.
[66] F. Dosio, P. Brusa, L. Cattel, Immunotoxins and anticancer drug conjugate assemblies: The role of the linkage between components, Toxins (Basel), 3 (2011) 848-883.
[67] N. Goodarzi, R. Varshochian, G. Kamalinia, F. Atyabi, R. Dinarvand, A review of polysaccharide cytotoxic drug conjugates for cancer therapy, Carbohydr. Polym., 92 (2013) 1280-1293.
[68] K. Akima, H. Ito, Y. Iwata, K. Matsuo, N. Watari, M. Yanagi, H. Hagi, K. Oshima, A. Yagita, Y. Atomi, I. Tatekawa, Evaluation of antitumor activities of hyaluronate binding antitumor drugs: Synthesis, characterization and antitumor activity, J. Drug Target., 4 (1996) 1-8.
[69] D. Coradini, C. Pellizzaro, G. Miglierini, M.G. Daidone, A. Perbellini, Hyaluronic acid as drug delivery for sodium butyrate: Improvement of the anti-proliferative activity on a breast-cancer cell line, Int. J. Cancer, 81 (1999) 411-416.
[70] A. Speranza, C. Pellizzaro, D. Coradini, Hyaluronic acid butyric esters in cancer therapy, Anticancer Drugs, 16 (2005) 373-379.
[71] C. Pellizzaro, A. Speranza, S. Zorzet, I. Crucil, G. Sava, I. Scarlata, S. Cantoni, M. Fedeli, D. Coradini, Inhibition of human pancreatic cell line MIA PaCa2 proliferation by HA-but, a hyaluronic butyric ester: A preliminary report, Pancreas, 36 (2008) e15-e23.
[72] D. Coradini, C. Pellizzaro, I. Scarlata, S. Zorzet, C. Garrovo, G. Abolafio, A. Speranza, M. Fedeli, S. Cantoni, G. Sava, M.G. Daidone, A. Perbellini, A novel retinoic/butyric hyaluronan ester for the treatment of acute promyelocytic leukemia: Preliminary preclinical results, Leukemia, 20 (2006) 785-792.
[73] T.M. Mekhail, M. Markman, Paclitaxel in cancer therapy, Expert Opin. Pharmacother., 3 (2002) 755-766.
[74] A. Rosato, A. Banzato, G. De Luca, D. Renier, F. Bettella, C. Pagano, G. Esposito, P. Zanovello, P. Bassi, HYTAD1-p20: A new paclitaxel-hyaluronic acid hydrosoluble bioconjugate for treatment of superficial bladder cancer, Urologic Oncology: Seminars and Original Investigations, 24 (2006) 207-215.
[75] A. Banzato, M. Rondina, L. Meléndez-Alafort, E. Zangoni, A. Nadali, D. Renier, G. Moschini, U. Mazzi, P. Zanovello, A. Rosato, Biodistribution imaging of a paclitaxel-hyaluronan bioconjugate, Nucl. Med. Biol., 36 (2009) 525-533.
[76] I. De Stefano, A. Battaglia, G.F. Zannoni, M.G. Prisco, A. Fattorossi, D. Travaglia, S. Baroni, D. Renier, G. Scambia, C. Ferlini, D. Gallo, Hyaluronic acid-paclitaxel: Effects of intraperitoneal administration against CD44(+) human ovarian cancer xenografts, Cancer Chemother. Pharmacol., 68 (2011) 107-116.
[77] A. Banzato, S. Bobisse, M. Rondina, D. Renier, F. Bettella, G. Esposito, L. Quintieri, L. Melendez-Alafort, U. Mazzi, P. Zanovello, A. Rosato, A paclitaxel-hyaluronan bioconjugateTargeting ovarian cancer affords a potent in vivo therapeutic activity, Clin. Cancer Res., 14 (2008) 3598-3606.
[78] M. Campisi, D. Renier, ONCOFID-P a hyaluronic acid paclitaxel conjugate for the treatment of refractory bladder cancer and peritoneal carcinosis, Current Bioactive Compounds, 7 (2011) 27-32.
[79] I.M. Montagner, A. Banzato, G. Zuccolotto, D. Renier, M. Campisi, P. Bassi, P. Zanovello, A. Rosato, Paclitaxel-hyaluronan hydrosoluble bioconjugate: Mechanism of action in human bladder cancer cell lines, Urologic Oncology: Seminars and Original Investigations, 31 (2013) 1261-1269.
[80] P.F. Bassi, A. Volpe, D. D'Agostino, G. Palermo, D. Renier, S. Franchini, A. Rosato, M. Racioppi, Paclitaxel-hyaluronic acid for intravesical therapy of bacillus calmette-gurin refractory carcinoma in situ of the bladder: Results of a phase i study, J. Urol., 185 (2011) 445-449.
[81] Y. Luo, G.D. Prestwich, Synthesis and selective cytotoxicity of a hyaluronic acid-antitumor bioconjugate, Bioconjug. Chem., 10 (1999) 755 - 763.

[82] E. Auzenne, S.C. Ghosh, M. Khodadadian, B. Rivera, D. Farquhar, R.E. Price, M. Ravoori, V. Kundra, R.S. Freedman, J. Klostergaard, Hyaluronic acid-paclitaxel: Antitumor efficacy against CD44(+) human ovarian carcinoma xenografts, Neoplasia, 9 (2007) 479-486.
[83] S.J. Lee, H.D. Han, R.L. Stone, J. Bottsford-Miller, D.Y. Shen, C. Lu, M. Nishimura, B. Zand, D. Thanapprapasr, N.B. Jennings, Y. Kang, J. Huang, W. Hu, A.K. Sood, S.C. Ghosh, E.J. Auzenne, A. Lopez-Araujo, J. Klostergaard, C.V. Pecot, Metronomic activity of CD44-targeted hyaluronic acid-paclitaxel in ovarian carcinoma, Clin. Cancer Res., 18 (2012) 4114 - 4121.
[84] C.E. Galer, D. Sano, S.C. Ghosh, J.H. Hah, E. Auzenne, A.N. Hamir, J.N. Myers, J. Klostergaard, Hyaluronic acid-paclitaxel conjugate inhibits growth of human squamous cell carcinomas of the head and neck via a hyaluronic acid-mediated mechanism, Oral Oncol., 47 (2011) 1039-1047.
[85] D. Xin, Y. Wang, J. Xiang, The use of amino acid linkers in the conjugation of paclitaxel with hyaluronic acid as drug delivery system: Synthesis, self-assembled property, drug release, and in vitro efficiency, Pharm. Res., 27 (2010) 380-389.
[86] R.K. Mittapalli, X. Liu, C.E. Adkins, M.I. Nounou, K.A. Bohn, T.B. Terrell, H.S. Qhattal, W.J. Geldenhuys, D. Palmieri, P.S. Steeg, Q.R. Smith, P.R. Lockman, Paclitaxel-hyaluronic nanoConjugates prolong overall survival in a preclinical brain metastases of breast cancer model, Mol. Cancer Ther., 12 (2013) 2389-2399.
[87] J.F. Pizzolato, L.B. Saltz, The camptothecins, Lancet, 361 (2003) 2235-2242.
[88] A. Serafino, M. Zonfrillo, F. Andreola, R. Psaila, L. Mercuri, N. Moroni, D. Renier, M. Campisi, C. Secchieri, P. Pierimarchi, CD44-targeting for antitumor drug delivery: a new SN-38-hyaluronan bioconjugate for locoregional treatment of peritoneal carcinomatosis, Curr. Cancer Drug Targets, 11 (2011) 572-585.
[89] G. Tringali, F. Bettella, M.C. Greco, M. Campisi, D. Renier, P. Navarra, Pharmacokinetic profile of Oncofid-S after intraperitoneal and intravenous administration in the rat, J. Pharm. Pharmacol., 64 (2012) 360-365.
[90] I.M. Montagner, A. Merlo, G. Zuccolotto, D. Renier, M. Campisi, G. Pasut, P. Zanovello, A. Rosato, Peritoneal tumor carcinomatosis: Pharmacological targeting with hyaluronan-based bioconjugates overcomes therapeutic indications of current drugs, PLoS One, 9 (2014).
[91] Z. Xu, W. Zheng, Z. Yin, Synthesis and optimization of a bifunctional hyaluronan-based camptothecin prodrug, Arch. Pharm., 347 (2014) 240-246.
[92] X. Yang, I. Dogan, V.R. Pannala, S. Kootala, J. Hilborn, D. Ossipov, A hyaluronic acid-camptothecin nanoprodrug with cytosolic mode of activation for targeting cancer, Polymer Chemistry, 4 (2013) 4621-4630.
[93] G. Takemura, H. Fujiwara, Doxorubicin-Induced Cardiomyopathy. From the Cardiotoxic Mechanisms to Management, Prog. Cardiovasc. Dis., 49 (2007) 330-352.
[94] S. Cai, S. Thati, T.R. Bagby, H.M. Diab, N.M. Davies, M.S. Cohen, M.L. Forrest M. Laird, Localized doxorubicin chemotherapy with a biopolymeric nanocarrier improves survival and reduces toxicity in xenografts of human breast cancer, J. Controlled Release, 146 (2010) 212-218.
[95] O.P. Oommen, J. Garousi, M. Sloff, O.P. Varghese, Tailored doxorubicin-hyaluronan conjugate as a potent anticancer glyco-drug: An alternative to prodrug approach, Macromol. Biosci., 14 (2014) 327-333.
[96] B.S. Chhikara, D. Mandal, K. Parang, Synthesis, anticancer activities, and cellular uptake studies of lipophilic derivatives of doxorubicin succinate, J. Med. Chem., 55 (2012) 1500-1510.
[97] B.S. Chhikara, N. St. Jean, D. Mandal, A. Kumar, K. Parang, Fatty acyl amide derivatives of doxorubicin: Synthesis and in vitro anticancer activities, Eur. J. Med. Chem., 46 (2011) 2037-2042.
[98] F. Kratz, INNO-206 (DOXO-EMCH), an albumin-binding prodrug of doxorubicin under development for Phase II studies, Current Bioactive Compounds, 7 (2011) 33-38.
[99] G.M. Dubowchik, S. Radia, H. Mastalerz, M.A. Walker, R.A. Firestone, H. Dalton King, S.J. Hofstead, D. Willner, S.J. Lasch, P.A. Trail, Doxorubicin immunoconjugates containing bivalent, lysosomally-Cleavable dipeptide linkages, Bioorg. Med. Chem. Lett., 12 (2002) 1529-1532.

[100] C. Fu, H. Li, N. Li, X. Miao, M. Xie, W. Du, L.-M. Zhang, Conjugating an anticancer drug onto thiolated hyaluronic acid by acid liable hydrazone linkage for its gelation and dual stimuli-response release, Carbohydr. Polym., 128 (2015) 163-170.
[101] K.M. Camacho, S. Kumar, S. Menegatti, D.R. Vogus, A.C. Anselmo, S. Mitragotri, Synergistic antitumor activity of camptothecin–doxorubicin combinations and their conjugates with hyaluronic acid, J. Controlled Release, 210 (2015) 198-207.
[102] B. Desoize, C. Madoulet, Particular aspects of platinum compounds used at present in cancer treatment, Crit. Rev. Oncol. Hematol., 42 (2002) 317-325.
[103] S. Cai, Y. Xie, T.R. Bagby, M.L. Forrest, M.S. Cohen, Intralymphatic Chemotherapy Using a Hyaluronan-Cisplatin Conjugate, J. Surg. Res., 147 (2008) 247 - 252.
[104] Y. Xie, K.L. Aillon, S. Cai, J.M. Christian, N.M. Davies, C.J. Berkland, M.L. Forrest, Pulmonary delivery of cisplatin-hyaluronan conjugates via endotracheal instillation for the treatment of lung cancer, Int. J. Pharm., 392 (2010) 156-163.
[105] Y.H. Quan, B. Kim, J.H. Park, Y. Choi, Y.H. Choi, H.K. Kim, Highly sensitive and selective anticancer effect by conjugated HA-cisplatin in non-small cell lung cancer overexpressed with CD44, Exp. Lung Res., 40 (2014) 475-484.
[106] S.M. Cohen, N. Rockefeller, R. Mukerji, D. Durham, M.L. Forrest, S. Cai, M.S. Cohen, Y. Shnayder, Efficacy and toxicity of peritumoral delivery of nanoconjugated cisplatin in an in vivo murine model of head and neck squamous cell carcinoma, JAMA Otolaryngology - Head and Neck Surgery, 139 (2013) 382-387.
[107] Q. Yang, D.J. Aires, S. Cai, G.R. Fraga, D. Zhang, C.Z. Li, M. Laird Forrest, In vivo efficacy of nano hyaluronan-conjugated cisplatin for treatment of murine melanoma, Journal of Drugs in Dermatology, 13 (2014) 283-287.
[108] R.O. Venable, D.R. Worley, D.L. Gustafson, R.J. Hansen, E.J. Ehrhart, S. Cai, M.S. Cohen, M. Laird Forrest, Effects of intratumoral administration of a hyaluronan-cisplatin nanoconjugate to five dogs with soft tissue sarcomas, Am. J. Vet. Res., 73 (2012) 1969-1973.
[109] S. Cai, A.A.B. Alhowyan, Q. Yang, W.C.M. Forrest, Y. Shnayder, M.L. Forrest, Cellular uptake and internalization of hyaluronan-based doxorubicin and cisplatin conjugates, J. Drug Target., 22 (2014) 648-657.
[110] S.M. Cohen, R. Mukerji, S. Cai, I. Damjanov, M.L. Forrest, M.S. Cohen, Subcutaneous delivery of nanoconjugated doxorubicin and cisplatin for locally advanced breast cancer demonstrates improved efficacy and decreased toxicity at lower doses than standard systemic combination therapy in vivo, Am. J. Surg., 202 (2011) 646-653.
[111] X. Ling, Y. Shen, R. Sun, M. Zhang, C. Li, J. Mao, J. Xing, C. Sun, J. Tu, Tumor-targeting delivery of hyaluronic acid-platinum(iv) nanoconjugate to reduce toxicity and improve survival, Polymer Chemistry, 6 (2015) 1541-1552.
[112] E.L. Ferguson, A.M.J. Alshame, D.W. Thomas, Evaluation of hyaluronic acid-protein conjugates for polymer masked-unmasked protein therapy, Int. J. Pharm., 402 (2010) 95-102.
[113] Y.H. Lee, H.Y. Yoon, J.M. Shin, G. Saravanakumar, K.H. Noh, K.H. Song, J.H. Jeon, D.W. Kim, K.M. Lee, K. Kim, I.C. Kwon, J.H. Park, T.W. Kim, A polymeric conjugate foreignizing tumor cells for targeted immunotherapy in vivo, J. Controlled Release, 199 (2015) 98-105.
[114] X. Pang, Z. Lu, H. Du, X. Yang, G. Zhai, Hyaluronic acid-quercetin conjugate micelles: synthesis, characterization, in vitro and in vivo evaluation, Colloids Surf. B. Biointerfaces, 123 (2014) 778-786.
[115] Y. Liu, J. Sun, P. Zhang, Z. He, Amphiphilic polysaccharide-hydrophobicized graft polymeric micelles for drug delivery nanosystems, Curr. Med. Chem., 18 (2011) 2638-2648.
[116] Y. Tian, S. Mao, Amphiphilic polymeric micelles as the nanocarrier for peroral delivery of poorly soluble anticancer drugs, Expert Opin Drug Deliv, 9 (2012) 687-700.
[117] E. Ruoslahti, S.N. Bhatia, M.J. Sailor, Targeting of drugs and nanoparticles to tumors, J. Cell Biol., 188 (2010) 759-768.

[118] L. Qiu, Z. Li, M. Qiao, M. Long, M. Wang, X. Zhang, C. Tian, D. Chen, Self-assembled pH-responsive hyaluronic acid-g-poly((L)-histidine) copolymer micelles for targeted intracellular delivery of doxorubicin, Acta Biomater., 10 (2014) 2024-2035.
[119] L. Qiu, M. Qiao, Q. Chen, C. Tian, M. Long, M. Wang, Z. Li, W. Hu, G. Li, L. Cheng, L. Cheng, H. Hu, X. Zhao, D. Chen, Enhanced effect of pH-sensitive mixed copolymer micelles for overcoming multidrug resistance of doxorubicin, Biomaterials, 35 (2014) 9877-9887.
[120] J. Li, M. Huo, J. Wang, J. Zhou, J.M. Mohammad, Y. Zhang, Q. Zhu, A.Y. Waddad, Q. Zhang, Redox-sensitive micelles self-assembled from amphiphilic hyaluronic acid-deoxycholic acid conjugates for targeted intracellular delivery of paclitaxel, Biomaterials, 33 (2012) 2310-2320.
[121] J. Li, T. Yin, L. Wang, L. Yin, J. Zhou, M. Huo, Biological evaluation of redox-sensitive micelles based on hyaluronic acid-deoxycholic acid conjugates for tumor-specific delivery of paclitaxel, Int. J. Pharm., 483 (2015) 38-48.
[122] T. Yin, L. Wang, L. Yin, J. Zhou, M. Huo, Co-delivery of hydrophobic paclitaxel and hydrophilic AURKA specific siRNA by redox-sensitive micelles for effective treatment of breast cancer, Biomaterials, 61 (2015) 10-25.
[123] Y. Shen, B. Wang, Y. Lu, A. Ouahab, Q. Li, J. Tu, A novel tumor-targeted delivery system with hydrophobized hyaluronic acid-spermine conjugates (HHSCs) for efficient receptor-mediated siRNA delivery, Int. J. Pharm., 414 (2011) 233-243.
[124] Y. Hu, J. Xie, Y.W. Tong, C.H. Wang, Effect of PEG conformation and particle size on the cellular uptake efficiency of nanoparticles with the HepG2 cells, J. Control. Release, 118 (2007) 7-17.
[125] D. Smejkalova, K. Nesporova, M. Hermannova, G. Huerta-Angeles, D. Cozikova, L. Vistejnova, B. Safrankova, J. Novotny, J. Kucerik, V. Velebny, Paclitaxel isomerisation in polymeric micelles based on hydrophobized hyaluronic acid, Int. J. Pharm., 466 (2014) 147-155.
[126] E. Saadat, M. Amini, M.R. Khoshayand, R. Dinarvand, F.A. Dorkoosh, Synthesis and optimization of a novel polymeric micelle based on hyaluronic acid and phospholipids for delivery of paclitaxel, in vitro and in-vivo evaluation, Int. J. Pharm., 475 (2014) 163-173.
[127] R.G. Thomas, M. Moon, S. Lee, Y.Y. Jeong, Paclitaxel loaded hyaluronic acid nanoparticles for targeted cancer therapy: In vitro and in vivo analysis, Int. J. Biol. Macromol., 72 (2014) 510-518.
[128] X. Liu, J. Zheng, J. Xu, H. Bai, C. Yi, J. Jiang, W. Mi, Method for preparing coumarin-grafted hyaluronic acid micelle emulsifier,CN102344501A, 2012
[129] Y. Liu, J. Sun, W. Cao, J. Yang, H. Lian, X. Li, Y. Sun, Y. Wang, S. Wang, Z. He, Dual targeting folate-conjugated hyaluronic acid polymeric micelles for paclitaxel delivery, Int. J. Pharm., 421 (2011) 160-169.
[130] Y. Liu, J. Sun, H. Lian, W. Cao, Y. Wang, Z. He, Folate and CD44 receptors dual-targeting hydrophobized hyaluronic acid paclitaxel-loaded polymeric micelles for overcoming multidrug resistance and improving tumor distribution, J. Pharm. Sci., 103 (2014) 1538-1547.
[131] Y. Liu, J. Sun, H. Lian, X. Li, W. Cao, L. Bai, Y. Wang, Z. He, Determination of paclitaxel in hyaluronic acid polymeric micelles in rat blood by protein precipitation-micelle breaking method: application to a pharmacokinetic study, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci., 935 (2013) 10-15.
[132] H. Lee, C.H. Ahn, T.G. Park, Poly[lactic-co-(glycolic acid)]-grafted hyaluronic acid copolymer micelle nanoparticles for target-specific delivery of doxorubicin, Macromol. Biosci., 9 (2009) 336-342.
[133] G.M. Son, H.Y. Kim, J.H. Ryu, C.W. Chu, D.H. Kang, S.B. Park, Y.I. Jeong, Self-assembled polymeric micelles based on hyaluronic acid-g-poly(D,L-lactide-co-glycolide) copolymer for tumor targeting, Int. J. Mol. Sci., 15 (2014) 16057-16068.
[134] G. Pitarresi, F.S. Palumbo, A. Albanese, C. Fiorica, P. Picone, G. Giammona, Self-assembled amphiphilic hyaluronic acid graft copolymers for targeted release of antitumoral drug, J. Drug Target., 18 (2010) 264-276.
[135] H. Yim, W. Park, D. Kim, T.M. Fahmy, K. Na, A self-assembled polymeric micellar immunomodulator for cancer treatment based on cationic amphiphilic polymers, Biomaterials, 35 (2014) 9912-9919.

[136] K. Li, H. Liu, W. Gao, M. Chen, Y. Zeng, J. Liu, L. Xu, D. Wu, Mulberry-like dual-drug complicated nanocarriers assembled with apogossypolone amphiphilic starch micelles and doxorubicin hyaluronic acid nanoparticles for tumor combination and targeted therapy, Biomaterials, 39 (2015) 131-144.
[137] 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.
[138] Y. Barenholz, Doxil® — The first FDA-approved nano-drug: Lessons learned, J. Controlled Release, 160 (2012) 117-134.
[139] M.L. Immordino, F. Dosio, L. Cattel, Stealth liposomes: Review of the basic science, rationale, and clinical applications, existing and potential, International Journal of Nanomedicine, 1 (2006) 297-315.
[140] T.M. Allen, P.R. Cullis, Liposomal drug delivery systems: From concept to clinical applications, Adv. Drug Del. Rev., 65 (2013) 36-48.
[141] P.P. Deshpande, S. Biswas, V.P. Torchilin, Current trends in the use of liposomes for tumor targeting, Nanomedicine, 8 (2013) 1509-1528.
[142] B. Ozpolat, A.K. Sood, G. Lopez-Berestein, Liposomal siRNA nanocarriers for cancer therapy, Adv. Drug Del. Rev., 66 (2014) 110-116.
[143] N. Yerushalmi, R. Margalit, Hyaluronic acid-modified bioadhesive liposomes as local drug depots: effects of cellular and fluid dynamics on liposome retention at target sites, Arch. Biochem. Biophys., 349 (1998) 21-26.
[144] E. Kim, J. Yang, J. Park, S. Kim, N.H. Kim, J.I. Yook, J.S. Suh, S. Haam, Y.M. Huh, Consecutive Targetable Smart Nanoprobe for Molecular Recognition of Cytoplasmic microRNA in Metastatic Breast Cancer, ACS Nano, 6 (2012) 8525-8535.
[145] H.S.S. Qhattal, X. Liu, Characterization of CD44-mediated cancer cell uptake and intracellular distribution of hyaluronan-grafted liposomes, Mol. Pharm., 8 (2011) 1233-1246.
[146] H.S.S. Qhattal, T. Hye, A. Alali, X. Liu, Hyaluronan polymer length, grafting density, and surface poly(ethylene glycol) coating influence in vivo circulation and tumor targeting of hyaluronan-grafted liposomes, ACS Nano, 8 (2014) 5423-5440.
[147] S. Arpicco, G. De Rosa, E. Fattal, Lipid-Based Nanovectors for Targeting of CD44-Overexpressing Tumor Cells, Journal of Drug Delivery, 2013 (2013) 8.
[148] S. Mizrahy, S.R. Raz, M. Hasgaard, H. Liu, N. Soffer-Tsur, K. Cohen, R. Dvash, D. Landsman-Milo, M.G.E.G. Bremer, S.M. Moghimi, D. Peer, Hyaluronan-coated nanoparticles: The influence of the molecular weight on CD44-hyaluronan interactions and on the immune response, J. Controlled Release, 156 (2011) 231-238.
[149] Y. Glucksam-Galnoy, T. Zor, R. Margalit, Hyaluronan-modified and regular multilamellar liposomes provide sub-cellular targeting to macrophages, without eliciting a pro-inflammatory response, J. Controlled Release, 160 (2012) 388-393.
[150] D. Landesman-Milo, M. Goldsmith, S. Leviatan Ben-Arye, B. Witenberg, E. Brown, S. Leibovitch, S. Azriel, S. Tabak, V. Morad, D. Peer, Hyaluronan grafted lipid-based nanoparticles as RNAi carriers for cancer cells, Cancer Lett., 334 (2013) 221-227.
[151] L. Gan, J. Wang, Y. Zhao, D. Chen, C. Zhu, J. Liu, Y. Gan, Hyaluronan-modified core–shell liponanoparticles targeting CD44-positive retinal pigment epithelium cells via intravitreal injection, Biomaterials, 34 (2013) 5978-5987.
[152] J. Lesley, V.C. Hascall, M. Tammi, R. Hyman, Hyaluronan binding by cell surface CD44, J. Biol. Chem., 275 (2000) 26967-26975.
[153] R.E. Eliaz, F.C. Szoka, Liposome-encapsulated doxorubicin targeted to CD44: A strategy to kill CD44-overexpressing tumor cells, Cancer Res., 61 (2001) 2592-2601.
[154] E. Dalla Pozza, C. Lerda, C. Costanzo, M. Donadelli, I. Dando, E. Zoratti, M.T. Scupoli, S. Beghelli, A. Scarpa, E. Fattal, S. Arpicco, M. Palmieri, Targeting gemcitabine containing liposomes to

CD44 expressing pancreatic adenocarcinoma cells causes an increase in the antitumoral activity, Biochim. Biophys. Acta, 1828 (2013) 1396-1404.
[155] D. Ruhela, S. Kivimäe, F.C. Szoka, Chemoenzymatic Synthesis of Oligohyaluronan–Lipid Conjugates, Bioconjug. Chem., 25 (2014) 718-723.
[156] C. Surace, S. Arpicco, A. Dufay-Wojcicki, V. Marsaud, C. Bouclier, D. Clay, L. Cattel, J.M. Renoir, E. Fattal, Lipoplexes Targeting the CD44 Hyaluronic Acid Receptor for Efficient Transfection of Breast Cancer Cells, Mol. Pharm., 6 (2009) 1062-1073.
[157] A.D. Wojcicki, H. Hillaireau, T.L. Nascimento, S. Arpicco, M. Taverna, S. Ribes, M. Bourge, V. Nicolas, A. Bochot, C. Vauthier, Hyaluronic acid-bearing lipoplexes: Physico-chemical characterization and in vitro targeting of the CD44 receptor, J. Controlled Release, (2012).
[158] J.H. Park, H.J. Cho, H.Y. Yoon, I.S. Yoon, S.H. Ko, J.S. Shim, J.H. Cho, J.H. Park, K. Kim, I.C. Kwon, D.D. Kim, Hyaluronic acid derivative-coated nanohybrid liposomes for cancer imaging and drug delivery, J. Controlled Release, 174 (2014) 98-108.
[159] S. Arpicco, C. Lerda, E. Dalla Pozza, C. Costanzo, N. Tsapis, B. Stella, M. Donadelli, I. Dando, E. Fattal, L. Cattel, M. Palmieri, Hyaluronic acid-coated liposomes for active targeting of gemcitabine, Eur. J. Pharm. Biopharm., 85 (2013) 373-380.
[160] N. Toriyabe, Y. Hayashi, M. Hyodo, H. Harashima, Synthesis and Evaluation of Stearylated Hyaluronic Acid for the Active Delivery of Liposomes to Liver Endothelial Cells, Biol. Pharm. Bull., 34 (2011) 1084-1089.
[161] D. Peer, R. Margalit, Loading mitomycin C inside long circulating hyaluronan targeted nano-liposomes increases its antitumor activity in three mice tumor models, Int. J. Cancer, 108 (2004) 780-789.
[162] D. Peer, R. Margalit, Tumor-targeted hyaluronan nanoliposomes increase the antitumor activity of liposomal doxorubicin in syngeneic and human xenograft mouse tumor models, Neoplasia, 6 (2004) 343-353.
[163] T. Jiang, Z. Zhang, Y. Zhang, H. Lv, J. Zhou, C. Li, L. Hou, Q. Zhang, Dual-functional liposomes based on pH-responsive cell-penetrating peptide and hyaluronic acid for tumor-targeted anticancer drug delivery, Biomaterials, 33 (2012) 9246-9258.
[164] L. Li, C.-H. Heldin, P. Heldin, Inhibition of platelet-derived growth factor-BB-induced receptor activation and fibroblast migration by hyaluronan activation of CD44, J. Biol. Chem., 281 (2006) 26512-26519.
[165] Z.R. Cohen, S. Ramishetti, N. Peshes-Yaloz, M. Goldsmith, A. Wohl, Z. Zibly, D. Peer, Localized RNAi therapeutics of chemoresistant grade IV glioma using hyaluronan-grafted lipid-based nanoparticles, ACS Nano, 9 (2015) 1581-1591.
[166] K. Simons, M.J. Gerl, Revitalizing membrane rafts: new tools and insights, Nature Reviews Molecular Cell Biology, 11 (2010) 688-699.
[167] N.M. Hooper, Detergent-insoluble glycosphingolipid/cholesterol-rich membrane domains, lipid rafts and caveolae (review), Mol. Membr. Biol., 16 (1999) 145-156.
[168] H. Ponta, L. Sherman, P.A. Herrlich, CD44: From adhesion molecules to signalling regulators, Nature Reviews Molecular Cell Biology, 4 (2003) 33-45.
[169] M. Long, S.-H. Huang, C.-H. Wu, G.M. Shackleford, A. Jong, Lipid raft/caveolae signaling is required for Cryptococcus neoformans invasion into human brain microvascular endothelial cells, J. Biomed. Sci., 19 (2012).
[170] T. Wrobel, P. Dziegiel, G. Mazur, M. Zabel, K. Kuliczkowski, A. Szuba, LYVE-1 expression on high endothelial venules (HEVs) of lymph nodes, Lymphology, 38 (2005) 107-110.
[171] E.N. Harris, S.V. Kyosseva, J.A. Weigel, P.H. Weigel, Expression, processing, and glycosaminoglycan binding activity of the recombinant human 315-kDa hyaluronic acid receptor for endocytosis (HARE), J. Biol. Chem., 282 (2007) 2785-2797.
[172] D.A. Ossipov, Nanostructured hyaluronic acid-based materials for active delivery to cancer, Expert Opinion on Drug Delivery, 7 (2010) 681 - 703.

[173] Y. Zhong, F. Meng, C. Deng, Z. Zhong, Ligand-directed active tumor-targeting polymeric nanoparticles for cancer chemotherapy, Biomacromolecules, 15 (2014) 1955-1969.
[174] O. Soula, R. Charvet, Nanoparticles comprising a hyaluronic acid-polylactide diblock copolymer,WO2015097242A1, 2015
[175] Y.I. Jeong, H. Kim do, C.W. Chung, J.J. Yoo, K.H. Choi, C.H. Kim, S.H. Ha, D.H. Kang, Self-assembled nanoparticles of hyaluronic acid/poly(DL-lactide-co-glycolide) block copolymer, Colloids Surf. B. Biointerfaces, 90 (2012) 28-35.
[176] J. Huang, H. Zhang, Y. Yu, Y. Chen, D. Wang, G. Zhang, G. Zhou, J. Liu, Z. Sun, D. Sun, Y. Lu, Y. Zhong, Biodegradable self-assembled nanoparticles of poly (D,L-lactide-co-glycolide)/hyaluronic acid block copolymers for target delivery of docetaxel to breast cancer, Biomaterials, 35 (2014) 550-566.
[177] A.K. Yadav, P. Mishra, A.K. Mishra, S. Jain, G.P. Agrawal, Development and characterization of hyaluronic acid-anchored PLGA nanoparticulate carriers of doxorubicin, Nanomedicine, 3 (2007) 246-257.
[178] A.K. Yadav, P. Mishra, S. Jain, A.K. Mishra, G.P. Agrawal, Preparation and characterization of HA-PEG-PCL intelligent core corona nanoparticles for delivery of doxorubicin, J. Drug Target., 16 (2008) 464-478.
[179] A.K. Yadav, A. Agarwal, G. Rai, P. Mishra, S. Jain, A.K. Mishra, H. Agrawal, G.P. Agrawal, Development and characterization of hyaluronic acid decorated PLGA nanoparticles for delivery of 5-fluorouracil, Drug Deliv., 17 (2010) 561-572.
[180] H.B. Nair, S. Huffman, P. Veerapaneni, N.B. Kirma, P. Binkley, R.P. Perla, D.B. Evans, R.R. Tekmal, Hyaluronic acid-bound letrozole nanoparticles restore sensitivity to letrozole-resistant xenograft tumors in mice, J Nanosci Nanotechnol, 11 (2011) 3789-3799.
[181] H.K. Park, S.J. Lee, J.S. Oh, S.G. Lee, Y.I. Jeong, H.C. Lee, Smart Nanoparticles Based on Hyaluronic Acid for Redox-Responsive and CD44 Receptor-Mediated Targeting of Tumor, Nanoscale Res Lett, 10 (2015) 981.
[182] K. Hu, H. Zhou, Y. Liu, Z. Liu, J. Liu, J. Tang, J. Li, J. Zhang, W. Sheng, Y. Zhao, Y. Wu, C. Chen, Hyaluronic acid functional amphipathic and redox-responsive polymer particles for the co-delivery of doxorubicin and cyclopamine to eradicate breast cancer cells and cancer stem cells, Nanoscale, 7 (2015) 8607-8618.
[183] H.S. Han, T. Thambi, K.Y. Choi, S. Son, H. Ko, M.C. Lee, D.G. Jo, Y.S. Chae, Y.M. Kang, J.Y. Lee, J.H. Park, Bioreducible shell-cross-linked hyaluronic acid nanoparticles for tumor-targeted drug delivery, Biomacromolecules, 16 (2015) 447-456.
[184] I. Youm, V. Agrahari, J.B. Murowchick, B.B. Youan, Uptake and cytotoxicity of docetaxel-loaded hyaluronic acid-grafted oily core nanocapsules in MDA-MB 231 cancer cells, Pharm. Res., 31 (2014) 2439-2452.
[185] K.K. Vangara, J.L. Liu, S. Palakurthi, Hyaluronic acid-decorated PLGA-PEG nanoparticles for targeted delivery of SN-38 to ovarian cancer, Anticancer Res., 33 (2013) 2425-2434.
[186] S. Zafar, L.M. Negi, A.K. Verma, V. Kumar, A. Tyagi, P. Singh, Z. Iqbal, S. Talegaonkar, Sterically stabilized polymeric nanoparticles with a combinatorial approach for multi drug resistant cancer: in vitro and in vivo investigations, Int. J. Pharm., 477 (2014) 454-468.
[187] M. He, Z. Zhao, L. Yin, C. Tang, C. Yin, Hyaluronic acid coated poly(butyl cyanoacrylate) nanoparticles as anticancer drug carriers, Int. J. Pharm., 373 (2009) 165-173.
[188] S. Abbad, C. Wang, A.Y. Waddad, H. Lv, J. Zhou, Preparation, in vitro and in vivo evaluation of polymeric nanoparticles based on hyaluronic acid-poly(butyl cyanoacrylate) and D-alpha-tocopheryl polyethylene glycol 1000 succinate for tumor-targeted delivery of morin hydrate, Int J Nanomedicine, 10 (2015) 305-320.
[189] C.S. Lee, K. Na, Photochemically triggered cytosolic drug delivery using pH-responsive hyaluronic acid nanoparticles for light-induced cancer therapy, Biomacromolecules, 15 (2014) 4228-4238.

[190] W. Wang, M. Xi, X. Duan, Y. Wang, F.E. Kong, Delivery of baicalein and paclitaxel using self-assembled nanoparticles: Synergistic antitumor effect in vitro and in vivo, International Journal of Nanomedicine, 10 (2015) 3737-3750.
[191] L. Battaglia, M. Gallarate, Lipid nanoparticles: state of the art, new preparation methods and challenges in drug delivery, Expert Opin Drug Deliv, 9 (2012) 497-508.
[192] H.J. Cho, H.Y. Yoon, H. Koo, S.H. Ko, J.S. Shim, J.H. Lee, K. Kim, I.C. Kwon, D.D. Kim, Self-assembled nanoparticles based on hyaluronic acid-ceramide (HA-CE) and Pluronic (R) for tumor-targeted delivery of docetaxel, Biomaterials, 32 (2011) 7181-7190.
[193] Y.J. Jin, U. Termsarasab, S.H. Ko, J.S. Shim, S. Chong, S.J. Chung, C.K. Shim, H.J. Cho, D.D. Kim, Hyaluronic acid derivative-based self-assembled nanoparticles for the treatment of melanoma, Pharm. Res., 29 (2012) 3443-3454.
[194] H.J. Cho, I.S. Yoon, H.Y. Yoon, H. Koo, Y.J. Jin, S.H. Ko, J.S. Shim, K. Kim, I.C. Kwon, D.D. Kim, Polyethylene glycol-conjugated hyaluronic acid-ceramide self-assembled nanoparticles for targeted delivery of doxorubicin, Biomaterials, 33 (2012) 1190-1200.
[195] J.H. Park, J.Y. Lee, U. Termsarasab, I.S. Yoon, S.H. Ko, J.S. Shim, H.J. Cho, D.D. Kim, Development of poly(lactic-co-glycolic) acid nanoparticles-embedded hyaluronic acid-ceramide-based nanostructure for tumor-targeted drug delivery, Int. J. Pharm., 473 (2014) 426-433.
[196] J.Y. Lee, H. Yang, I.S. Yoon, S.B. Kim, S.H. Ko, J.S. Shim, S.H. Sung, H.J. Cho, D.D. Kim, Nanocomplexes based on amphiphilic hyaluronic acid derivative and polyethylene glycol-lipid for ginsenoside rg3 delivery, J. Pharm. Sci., 103 (2014) 3254-3262.
[197] K.Y. Choi, H.Y. Yoon, J.-H. Kim, S.M. Bae, R.-W. Park, Y.M. Kang, I.-S. Kim, I.C. Kwon, K. Choi, S.Y. Jeong, K. Kim, J.H. Park, Smart Nanocarrier Based on PEGylated Hyaluronic Acid for Cancer Therapy, ACS Nano, 5 (2011) 8591-8599.
[198] H.Y. Yoon, H. Koo, K.Y. Choi, S.J. Lee, K. Kim, I.C. Kwon, J.F. Leary, K. Park, S.H. Yuk, J.H. Park, K. Choi, Tumor-targeting hyaluronic acid nanoparticles for photodynamic imaging and therapy, Biomaterials, 33 (2012) 3980-3989.
[199] K.Y. Choi, E.J. Jeon, H.Y. Yoon, B.S. Lee, J.H. Na, K.H. Min, S.Y. Kim, S.J. Myung, S. Lee, X. Chen, I.C. Kwon, K. Choi, S.Y. Jeong, K. Kim, J.H. Park, Theranostic nanoparticles based on PEGylated hyaluronic acid for the diagnosis, therapy and monitoring of colon cancer, Biomaterials, 33 (2012) 6186-6193.
[200] G. Liu, K.Y. Choi, A. Bhirde, M. Swierczewska, J. Yin, S.W. Lee, J.H. Park, J.I. Hong, J. Xie, G. Niu, D.O. Kiesewetter, S. Lee, X. Chen, Sticky nanoparticles: a platform for siRNA delivery by a bis(zinc(II) dipicolylamine)-functionalized, self-assembled nanoconjugate, Angew. Chem. Int. Ed. Engl., 51 (2012) 445-449.
[201] H.Y. Yoon, H. Koo, K.Y. Choi, I. Chan Kwon, K. Choi, J.H. Park, K. Kim, Photo-crosslinked hyaluronic acid nanoparticles with improved stability for invivo tumor-targeted drug delivery, Biomaterials, 34 (2013) 5273-5280.
[202] H.S. Han, J. Lee, H.R. Kim, S.Y. Chae, M. Kim, G. Saravanakumar, H.Y. Yoon, D.G. You, H. Ko, K. Kim, I.C. Kwon, J.C. Park, J.H. Park, Robust PEGylated hyaluronic acid nanoparticles as the carrier of doxorubicin: mineralization and its effect on tumor targetability in vivo, J. Control. Release, 168 (2013) 105-114.
[203] Y. Zhong, J. Zhang, R. Cheng, C. Deng, F. Meng, F. Xie, Z. Zhong, Reversibly crosslinked hyaluronic acid nanoparticles for active targeting and intelligent delivery of doxorubicin to drug resistant CD44+ human breast tumor xenografts, J. Control. Release, 205 (2015) 144-154.
[204] J. Yao, L. Zhang, J. Zhou, H. Liu, Q. Zhang, Efficient simultaneous tumor targeting delivery of all-trans retinoid acid and paclitaxel based on hyaluronic acid-based multifunctional nanocarrier, Mol. Pharm., 10 (2013) 1080-1091.
[205] J. Yao, Y. Li, X. Sun, F.Z. Dahmani, H. Liu, J. Zhou, Nanoparticle delivery and combination therapy of gambogic acid and all-trans retinoic acid, Int J Nanomedicine, 9 (2014) 3313-3324.

[206] D. Liang, A.T. Wang, Z.Z. Yang, Y.J. Liu, X.R. Qi, Enhance cancer cell recognition and overcome drug resistance using hyaluronic acid and α-tocopheryl succinate based multifunctional nanoparticles, Mol. Pharm., 12 (2015) 2189-2202.
[207] L. Zhang, J. Yao, J. Zhou, T. Wang, Q. Zhang, Glycyrrhetinic acid-graft-hyaluronic acid conjugate as a carrier for synergistic targeted delivery of antitumor drugs, Int. J. Pharm., 441 (2013) 654-664.
[208] O. Mezghrani, Y. Tang, X. Ke, Y. Chen, D. Hu, J. Tu, L. Zhao, N. Bourkaib, Hepatocellular carcinoma dually-targeted nanoparticles for reduction triggered intracellular delivery of doxorubicin, Int. J. Pharm., 478 (2015) 530-539.
[209] S. Ganesh, A.K. Iyer, D.V. Morrissey, M.M. Amiji, Hyaluronic acid based self-assembling nanosystems for CD44 target mediated siRNA delivery to solid tumors, Biomaterials, 34 (2013) 3489-3502.
[210] S. Ganesh, A.K. Iyer, F. Gattacceca, D.V. Morrissey, M.M. Amiji, In vivo biodistribution of siRNA and cisplatin administered using CD44-targeted hyaluronic acid nanoparticles, J. Controlled Release, 172 (2013) 699-706.
[211] S. Song, F. Chen, H. Qi, F. Li, T. Xin, J. Xu, T. Ye, N. Sheng, X. Yang, W. Pan, Multifunctional Tumor-Targeting Nanocarriers Based on Hyaluronic Acid-Mediated and pH-Sensitive Properties for Efficient Delivery of Docetaxel, Pharm. Res., 31 (2014) 1032-1045.
[212] S. Song, H. Qi, J. Xu, P. Guo, F. Chen, F. Li, X. Yang, N. Sheng, Y. Wu, W. Pan, Hyaluronan-based nanocarriers with CD44-overexpressed cancer cell targeting, Pharm. Res., 31 (2014) 2988-3005.
[213] T.H. Tran, J.Y. Choi, T. Ramasamy, D.H. Truong, C.N. Nguyen, H.G. Choi, C.S. Yong, J.O. Kim, Hyaluronic acid-coated solid lipid nanoparticles for targeted delivery of vorinostat to CD44 overexpressing cancer cells, Carbohydr. Polym., 114 (2014) 407-415.
[214] H. Shen, S. Shi, Z. Zhang, T. Gong, X. Sun, Coating Solid Lipid Nanoparticles with Hyaluronic Acid Enhances Antitumor Activity against Melanoma Stem-like Cells, Theranostics, 5 (2015) 755-771.
[215] T. Ramasamy, T.H. Tran, J.Y. Choi, H.J. Cho, J.H. Kim, C.S. Yong, H.G. Choi, J.O. Kim, Layer-by-layer coated lipid-polymer hybrid nanoparticles designed for use in anticancer drug delivery, Carbohydr. Polym., 102 (2014) 653-661.
[216] D. Liu, Z. Liu, L. Wang, C. Zhang, N. Zhang, Nanostructured lipid carriers as novel carrier for parenteral delivery of docetaxel, Colloids Surf. B. Biointerfaces, 85 (2011) 262-269.
[217] L.M. Negi, M. Jaggi, V. Joshi, K. Ronodip, S. Talegaonkar, Hyaluronic acid decorated lipid nanocarrier for MDR modulation and CD-44 targeting in colon adenocarcinoma, Int. J. Biol. Macromol., 72 (2015) 569-574.
[218] L.M. Negi, S. Talegaonkar, M. Jaggi, A.K. Verma, R. Verma, S. Dobhal, V. Kumar, Surface engineered nanostructured lipid carriers for targeting MDR tumor: Part I. Synthesis, characterization and in vitro investigation, Colloids Surf B Biointerfaces, 123 (2014) 600-609.
[219] L.M. Negi, S. Talegaonkar, M. Jaggi, A.K. Verma, R. Verma, S. Dobhal, V. Kumar, Surface engineered nanostructured lipid carriers for targeting MDR tumor: Part II. In vivo biodistribution, pharmacodynamic and hematological toxicity studies, Colloids Surf. B. Biointerfaces, 123 (2014) 610-615.
[220] C.Y. Qu, M. Zhou, Y.W. Chen, M.M. Chen, F. Shen, L.M. Xu, Engineering of lipid prodrug-based, hyaluronic acid-decorated nanostructured lipid carriers platform for 5-fluorouracil and cisplatin combination gastric cancer therapy, International Journal of Nanomedicine, 10 (2015) 3911-3920.
[221] X.Y. Yang, Y.X. Li, M. Li, L. Zhang, L.X. Feng, N. Zhang, Hyaluronic acid-coated nanostructured lipid carriers for targeting paclitaxel to cancer, Cancer Lett., 334 (2013) 338-345.
[222] I. Rivkin, K. Cohen, J. Koffler, D. Melikhov, D. Peer, R. Margalit, Paclitaxel-clusters coated with hyaluronan as selective tumor-targeted nanovectors, Biomaterials, 31 (2010) 7106-7114.
[223] G. Bachar, K. Cohen, R. Hod, R. Feinmesser, A. Mizrachi, T. Shpitzer, O. Katz, D. Peer, Hyaluronan-grafted particle clusters loaded with Mitomycin C as selective nanovectors for primary head and neck cancers, Biomaterials, 32 (2011) 4840-4848.

[224] D. Peer, A daunting task: manipulating leukocyte function with RNAi, Immunol. Rev., 253 (2013) 185-197.
[225] K. Cohen, R. Emmanuel, E. Kisin-Finfer, D. Shabat, D. Peer, Modulation of drug resistance in ovarian adenocarcinoma using chemotherapy entrapped in hyaluronan-grafted nanoparticle clusters, ACS Nano, 8 (2014) 2183-2195.
[226] S. Mizrahy, M. Goldsmith, S. Leviatan-Ben-Arye, E. Kisin-Finfer, O. Redy, S. Srinivasan, D. Shabat, B. Godin, D. Peer, Tumor targeting profiling of hyaluronan-coated lipid based-nanoparticles, Nanoscale, 6 (2014) 3742-3752.
[227] A.V. Kabanov, S.V. Vinogradov, Nanogels as pharmaceutical carriers: finite networks of infinite capabilities, Angew. Chem. Int. Ed. Engl., 48 (2009) 5418-5429.
[228] Y. Wen, J.K. Oh, Recent strategies to develop polysaccharide-based nanomaterials for biomedical applications, Macromol. Rapid Commun., 35 (2014) 1819-1832.
[229] A. Jain, S.K. Jain, In vitro and cell uptake studies for targeting of ligand anchored nanoparticles for colon tumors, Eur. J. Pharm. Sci., 35 (2008) 404-416.
[230] A. Jain, S.K. Jain, N. Ganesh, J. Barve, A.M. Beg, Design and development of ligand-appended polysaccharidic nanoparticles for the delivery of oxaliplatin in colorectal cancer, Nanomed. Nanotechnol. Biol. Med., 6 (2010) 179-190.
[231] X. Deng, M. Cao, J. Zhang, K. Hu, Z. Yin, Z. Zhou, X. Xiao, Y. Yang, W. Sheng, Y. Wu, Y. Zeng, Hyaluronic acid-chitosan nanoparticles for co-delivery of MiR-34a and doxorubicin in therapy against triple negative breast cancer, Biomaterials, 35 (2014) 4333-4344.
[232] E. Kim, J. Yang, H.O. Kim, Y. An, E.K. Lim, G. Lee, T. Kwon, J.H. Cheong, J.S. Suh, Y.M. Huh, S. Haam, Hyaluronic acid receptor-targetable imidazolized nanovectors for induction of gastric cancer cell death by RNA interference, Biomaterials, 34 (2013) 4327-4338.
[233] X. Yang, A.K. Iyer, A. Singh, L. Milane, E. Choy, F.J. Hornicek, M.M. Amiji, Z. Duan, Cluster of Differentiation 44 Targeted Hyaluronic Acid Based Nanoparticles for MDR1 siRNA Delivery to Overcome Drug Resistance in Ovarian Cancer, Pharm. Res., 32 (2015) 2097-2109.
[234] X. Yang, A.K. Iyer, A. Singh, E. Choy, F.J. Hornicek, M.M. Amiji, Z. Duan, MDR1 siRNA loaded hyaluronic acid-based CD44 targeted nanoparticle systems circumvent paclitaxel resistance in ovarian cancer, Sci. Rep., 5 (2015) 8509.
[235] W. Zhang, Q. Cheng, S. Guo, D. Lin, P. Huang, J. Liu, T. Wei, L. Deng, Z. Liang, X.J. Liang, A. Dong, Gene transfection efficacy and biocompatibility of polycation/DNA complexes coated with enzyme degradable PEGylated hyaluronic acid, Biomaterials, 34 (2013) 6495-6503.
[236] R. Mo, T. Jiang, R. Disanto, W. Tai, Z. Gu, ATP-triggered anticancer drug delivery, Nature Communications, 5 (2014).
[237] A.M. Al-Ghananeem, A.H. Malkawi, Y.M. Muammer, J.M. Balko, E.P. Black, W. Mourad, E. Romond, Intratumoral delivery of Paclitaxel in solid tumor from biodegradable hyaluronan nanoparticle formulations, AAPS PharmSciTech, 10 (2009) 410-417.
[238] C. Yang, X. Wang, X. Yao, Y. Zhang, W. Wu, X. Jiang, Hyaluronic acid nanogels with enzyme-sensitive cross-linking group for drug delivery, J. Control. Release, 205 (2015) 206-217.
[239] W. Park, K.S. Kim, B.C. Bae, Y.H. Kim, K. Na, Cancer cell specific targeting of nanogels from acetylated hyaluronic acid with low molecular weight, Eur. J. Pharm. Sci., 40 (2010) 367-375.
[240] X. Wei, T.H. Senanayake, A. Bohling, S.V. Vinogradov, Targeted nanogel conjugate for improved stability and cellular permeability of curcumin: synthesis, pharmacokinetics, and tumor growth inhibition, Mol. Pharm., 11 (2014) 3112-3122.
[241] K. Maier-Hauff, F. Ulrich, D. Nestler, H. Niehoff, P. Wust, B. Thiesen, H. Orawa, V. Budach, A. Jordan, Efficacy and safety of intratumoral thermotherapy using magnetic iron-oxide nanoparticles combined with external beam radiotherapy on patients with recurrent glioblastoma multiforme, J. Neurooncol., 103 (2011) 317-324.
[242] G.F. Paciotti, L. Tamarkin, J. Zhao, S. Cao, S. Cao, D.G.I. Kingston, Abstract 3695: CYT-20000: Targeted and temporal delivery of a VDA and chemotherapy by a single gold nanodrug, Cancer Res., 70 (2010) 3695.

[243] T. Pellegrino, L. Manna, S. Kudera, T. Liedl, D. Koktysh, A.L. Rogach, S. Keller, J. RÃ¤dler, G. Natile, W.J. Parak, Hydrophobic nanocrystals coated with an amphiphilic polymer shell: A general route to water soluble nanocrystals, Nano Lett., 4 (2004) 703-707.
[244] J. Kim, K.S. Kim, G. Jiang, H. Kang, S. Kim, B.-S. Kim, M.H. Park, S.K. Hahn, In vivo real-time bioimaging of hyaluronic acid derivatives using quantum dots, Biopolymers, 89 (2008) 1144-1153.
[245] Kim, W. Hur, S.-J. Park, S.W. Hong, J.E. Choi, E.J. Goh, S.K. Yoon, S.K. Hahn, Bioimaging for targeted delivery of hyaluronic Acid derivatives to the livers in cirrhotic mice using quantum dots, ACS nano, 4 (2010) 3005 - 3014.
[246] H. Wang, H. Sun, H. Wei, P. Xi, S. Nie, Q. Ren, Biocompatible hyaluronic acid polymer-coated quantum dots for CD44+ cancer cell-targeted imaging, J. Nanopart. Res., 16 (2014).
[247] H. Xu, J. He, Y. Zhang, L. Fan, Y. Zhao, T. Xu, Z. Nie, X. Li, Z. Huang, B. Lu, P. Xu, Synthesis and in vitro evaluation of a hyaluronic acid–quantum dots–melphalan conjugate, Carbohydr. Polym., 121 (2015) 132-139.
[248] O.S. Muddineti, B. Ghosh, S. Biswas, Current trends in using polymer coated gold nanoparticles for cancer therapy, Int. J. Pharm., 484 (2015) 252-267.
[249] H. Lee, K. Lee, I.K. Kim, T.G. Park, Synthesis, characterization, and in vivo diagnostic applications of hyaluronic acid immobilized gold nanoprobes, Biomaterials, 29 (2008) 4709-4718.
[250] S.H. Kang, M. Nafiujjaman, M. Nurunnabi, L. Li, H.A. Khan, K.J. Cho, K.M. Huh, Y.-k. Lee, Hybrid photoactive nanomaterial composed of gold nanoparticles, pheophorbide-A and hyaluronic acid as a targeted bimodal phototherapy, Macromolecular Research, 23 (2015) 474-484.
[251] M.H. El-Dakdouki, D.C. Zhu, K. El-Boubbou, M. Kamat, J. Chen, W. Li, X. Huang, Development of Multifunctional Hyaluronan-Coated Nanoparticles for Imaging and Drug Delivery to Cancer Cells, Biomacromolecules, 13 (2012) 1144-1151.
[252] J. Li, Y. He, W. Sun, Y. Luo, H. Cai, Y. Pan, M. Shen, J. Xia, X. Shi, Hyaluronic acid-modified hydrothermally synthesized iron oxide nanoparticles for targeted tumor MR imaging, Biomaterials, 35 (2014) 3666-3677.
[253] R.G. Thomas, M.J. Moon, H. Lee, A.R.K. Sasikala, C.S. Kim, I.K. Park, Y.Y. Jeong, Hyaluronic acid conjugated superparamagnetic iron oxide nanoparticle for cancer diagnosis and hyperthermia therapy, Carbohydr. Polym., 131 (2015) 439-446.
[254] D. Chen, C.A. Dougherty, K. Zhu, H. Hong, Theranostic applications of carbon nanomaterials in cancer: Focus on imaging and cargo delivery, J. Controlled Release, 210 (2015) 230-245.
[255] J. Bartelmess, S.J. Quinn, S. Giordani, Carbon nanomaterials: Multi-functional agents for biomedical fluorescence and Raman imaging, Chem. Soc. Rev., 44 (2015) 4672-4698.
[256] X. Yang, A. Ebrahimi, J. Li, Q. Cui, Fullerene-biomolecule conjugates and their biomedicinal applications, International Journal of Nanomedicine, 9 (2014) 77-92.
[257] D.S. Kwag, K. Park, K.T. Oh, E.S. Lee, Hyaluronated fullerenes with photoluminescent and antitumoral activity, Chem. Commun., 49 (2013) 282-284.
[258] S. Kim, J. Park, Y.S. Youn, K.T. Oh, J.H. Bae, E.S. Lee, Hoechst 33258-conjugated hyaluronated fullerene for efficient photodynamic tumor therapy and necrotic tumor targeting, J. Bioact. Compat. Polym., 30 (2015) 275-288.
[259] H. Ali‐Boucetta, K. Kostarelos, Carbon nanotubes in medicine & biology − Therapy and diagnostics, Adv. Drug Del. Rev., 65 (2013) 1897-1898.
[260] R. Singh, S.V. Torti, Carbon nanotubes in hyperthermia therapy, Adv. Drug Del. Rev., 65 (2013) 2045-2060.
[261] B.S. Wong, S.L. Yoong, A. Jagusiak, T. Panczyk, H.K. Ho, W.H. Ang, G. Pastorin, Carbon nanotubes for delivery of small molecule drugs, Adv. Drug Del. Rev., 65 (2013) 1964-2015.
[262] R. Marega, M. Bergamin, V. Aroulmoji, F. Dinon, M. Prato, E. Murano, Hyaluronan-Carbon Nanotube Derivatives: Synthesis, Conjugation with Model Drugs, and DOSY NMR Characterization, Eur. J. Org. Chem., 2011 (2011) 5617-5625, S5617/5611-S5617/5610.

[263] S.R. Datir, M. Das, R.P. Singh, S. Jain, Hyaluronate Tethered, "Smart" Multiwalled Carbon Nanotubes for Tumor-Targeted Delivery of Doxorubicin, Bioconjugate Chem., 23 (2012) 2201-2213.
[264] J. Shi, R. Ma, L. Wang, J. Zhang, R. Liu, L. Li, Y. Liu, L. Hou, X. Yu, J. Gao, Z. Zhang, The application of hyaluronic acid-derivatized carbon nanotubes in hematoporphyrin monomethyl ether-based photodynamic therapy for in vivo and in vitro cancer treatment, Int. J. Nanomed., 8 (2013) 2361-2373.
[265] Y. Mo, H. Wang, J. Liu, Y. Lan, R. Guo, Y. Zhang, W. Xue, Y. Zhang, Controlled release and targeted delivery to cancer cells of doxorubicin from polysaccharide-functionalised single-walled carbon nanotubes, Journal of Materials Chemistry B, 3 (2015) 1846-1855.
[266] H.J. Yao, Y.G. Zhang, L. Sun, Y. Liu, The effect of hyaluronic acid functionalized carbon nanotubes loaded with salinomycin on gastric cancer stem cells, Biomaterials, 35 (2014) 9208-9223.
[267] X. Cao, L. Tao, S. Wen, W. Hou, X. Shi, Hyaluronic acid-modified multiwalled carbon nanotubes for targeted delivery of doxorubicin into cancer cells, Carbohydr. Res., 405 (2015) 70-77.
[268] M. Swierczewska, K.Y. Choi, E.L. Mertz, X. Huang, F. Zhang, L. Zhu, H.Y. Yoon, J.H. Park, A. Bhirde, S. Lee, X. Chen, A Facile, One-Step Nanocarbon Functionalization for Biomedical Applications, Nano Lett., 12 (2012) 3613-3620.
[269] R. Dvash, A. Khatchatouriants, L.J. Solmesky, P.P. Wibroe, M. Weil, S.M. Moghimi, D. Peer, Structural profiling and biological performance of phospholipid-hyaluronan functionalized single-walled carbon nanotubes, J. Controlled Release, 170 (2013) 295-305.
[270] A.A. Bhirde, B.V. Chikkaveeraiah, A. Srivatsan, G. Niu, A.J. Jin, A. Kapoor, Z. Wang, S. Patel, V. Patel, A.M. Gorbach, R.D. Leapman, J.S. Gutkind, A.R. Hight Walker, X. Chen, Targeted therapeutic nanotubes influence the viscoelasticity of cancer cells to overcome drug resistance, ACS Nano, 8 (2014) 4177-4189.
[271] X.T. Zheng, A. Ananthanarayanan, K.Q. Luo, P. Chen, Glowing graphene quantum dots and carbon dots: Properties, syntheses, and biological applications, Small, 11 (2015) 1620-1636.
[272] F. Li, S.-J. Park, D. Ling, W. Park, J.Y. Han, K. Na, K. Char, Hyaluronic acid-conjugated graphene oxide/photosensitizer nanohybrids for cancer targeted photodynamic therapy, J. Mater. Chem. B, 1 (2013) 1678-1686.
[273] L. Hou, Q. Feng, Y. Wang, H. Zhang, G. Jiang, X. Yang, J. Ren, X. Zhu, Y. Shi, Z. Zhang, Multifunctional nanosheets based on hyaluronic acid modified graphene oxide for tumor-targeting chemophotothermal therapy, J. Nanopart. Res., 17 (2015).
[274] H. Wu, H. Shi, Y. Wang, X. Jia, C. Tang, J. Zhang, S. Yang, Hyaluronic acid conjugated graphene oxide for targeted drug delivery, Carbon, 69 (2014) 379-389.
[275] E. Byun, H. Lee, Enhanced loading efficiency and sustained release of doxorubicin from hyaluronic acid/graphene oxide composite hydrogels by a mussel-inspired catecholamine, J Nanosci Nanotechnol, 14 (2014) 7395-7401.
[276] A. Al Nahain, J.-E. Lee, I. In, H. Lee, K.D. Lee, J.H. Jeong, S.Y. Park, Target Delivery and Cell Imaging Using Hyaluronic Acid-Functionalized Graphene Quantum Dots, Mol. Pharmaceutics, 10 (2013) 3736-3744.
[277] A. Baeza, M. Colilla, M. Vallet-Regí, Advances in mesoporous silica nanoparticles for targeted stimuli-responsive drug delivery, Expert Opinion on Drug Delivery, 12 (2015) 319-337.
[278] Z. Chen, Z. Li, Y. Lin, M. Yin, J. Ren, X. Qu, Bioresponsive hyaluronic acid-capped mesoporous silica nanoparticles for targeted drug delivery, Chemistry - A European Journal, 19 (2013) 1778-1783.
[279] Q. Zhao, J. Liu, W. Zhu, C. Sun, D. Di, Y. Zhang, P. Wang, Z. Wang, S. Wang, Dual-stimuli responsive hyaluronic acid-conjugated mesoporous silica for targeted delivery to CD44-overexpressing cancer cells, Acta Biomater., (2015).
[280] X. Wang, H. Chen, K. Zhang, M. Ma, F. Li, D. Zeng, S. Zheng, Y. Chen, L. Jiang, H. Xu, J. Shi, An intelligent nanotheranostic agent for targeting, redox-responsive ultrasound imaging, and imaging-guided high-intensity focused ultrasound synergistic therapy, Small, 10 (2014) 1403-1411.

[281] M.H. El-Dakdouki, E. Puré, X. Huang, Development of drug loaded nanoparticles for tumor targeting. Part 1: Synthesis, characterization, and biological evaluation in 2D cell cultures, Nanoscale, 5 (2013) 3895-3903.
[282] M.H. El-Dakdouki, E. Puré, X. Huang, Development of drug loaded nanoparticles for tumor targeting. Part 2: Enhancement of tumor penetration through receptor mediated transcytosis in 3D tumor models, Nanoscale, 5 (2013) 3904-3911.
[283] L. Huang, L. Ao, W. Wang, D. Hu, Z. Sheng, W. Su, Multifunctional magnetic silica nanotubes for MR imaging and targeted drug delivery, Chem. Commun., 51 (2015) 3923-3926.
[284] J. Kreuter, Nanoparticles and microparticles for drug and vaccine delivery, J. Anat., 189 (Pt 3) (1996) 503-505.
[285] K. Laeschke, Biocompatibility of microparticles into soft tissue fillers, Semin. Cutan. Med. Surg., 23 (2004) 214-217.
[286] M.N. Aly, Intra-articular drug delivery: a fast growing approach, Recent Pat Drug Deliv Formul, 2 (2008) 231-237.
[287] S.D. Li, S.B. Howell, CD44-targeted microparticles for delivery of cisplatin to peritoneal metastases, Mol. Pharm., 7 (2010) 280-290.
[288] L. Genc, S. Buyuktiryaki, Preparation and characterization of methotrexate-loaded microcapsules, Pharm. Dev. Technol., 19 (2014) 42-47.
[289] S. Harada, S. Ehara, K. Ishii, H. Yamazaki, S. Matsuyama, T. Sato, S. Oikawa, T. Kamiya, K. Arakawa, W. Yokota, K. Sera, J. Ito, Targeted delivery of chemotherapeutic agents using improved radiosensitive liquid core microcapsules and assessment of their antitumor effect, Int. J. Radiat. Oncol. Biol. Phys., 75 (2009) 455-462.
[290] C.J. Needham, S.R. Shah, P.M. Mountziaris, F.K. Kasper, A.G. Mikos, Modulation of polyplex release from biodegradable microparticles through poly(ethylenimine) modification and varying loading concentration, Pharm. Res., 31 (2014) 77-85.
[291] C.C. DeMerlis, D.R. Schoneker, Review of the oral toxicity of polyvinyl alcohol (PVA), Food Chem. Toxicol., 41 (2003) 319-326.
[292] B. Cerroni, E. Chiessi, S. Margheritelli, L. Oddo, G. Paradossi, Polymer shelled microparticles for a targeted doxorubicin delivery in cancer therapy, Biomacromolecules, 12 (2011) 593-601.
[293] C.M. Nimmo, M.S. Shoichet, Regenerative biomaterials that "click": simple, aqueous-based protocols for hydrogel synthesis, surface immobilization, and 3D patterning, Bioconjug. Chem., 22 (2011) 2199-2209.
[294] S.G. Kupal, B. Cerroni, S.V. Ghugare, E. Chiessi, G. Paradossi, Biointerface properties of core-shell poly(vinyl alcohol)-hyaluronic acid microgels based on chemoselective chemistry, Biomacromolecules, 13 (2012) 3592-3601.
[295] U. Anderegg, J.C. Simon, M. Averbeck, More than just a filler - the role of hyaluronan for skin homeostasis, Exp. Dermatol., 23 (2014) 295-303.
[296] R.D. Price, S. Myers, I.M. Leigh, H.A. Navsaria, The role of hyaluronic acid in wound healing: Assessment of clinical evidence, Am. J. Clin. Dermatol., 6 (2005) 393-402.
[297] T.M. Tamer, Hyaluronan and synovial joint: Function, distribution and healing, Interdiscip. Toxicol., 6 (2013) 111-125.
[298] G.D. Prestwich, Hyaluronic acid-based clinical biomaterials derived for cell and molecule delivery in regenerative medicine, J. Control. Release, 155 (2011) 193-199.
[299] J.A. Burdick, G.D. Prestwich, Hyaluronic acid hydrogels for biomedical applications, Adv. Mater., 23 (2011) H41-56.
[300] S. Emoto, H. Yamaguchi, T. Kamei, H. Ishigami, T. Suhara, Y. Suzuki, T. Ito, J. Kitayama, T. Watanabe, Intraperitoneal administration of cisplatin via an in situ cross-linkable hyaluronic acid-based hydrogel for peritoneal dissemination of gastric cancer, Surg. Today, 44 (2014) 919-926.
[301] J.P. Chen, Y.L. Leu, C.L. Fang, C.H. Chen, J.Y. Fang, Thermosensitive hydrogels composed of hyaluronic acid and gelatin as carriers for the intravesical administration of cisplatin, J. Pharm. Sci., 100 (2011) 655-666.

[302] Y.Y. Chen, H.C. Wu, J.S. Sun, G.C. Dong, T.W. Wang, Injectable and thermoresponsive self-assembled nanocomposite hydrogel for long-term anticancer drug delivery, Langmuir, 29 (2013) 3721-3729.
[303] H.J. Jhan, J.J. Liu, Y.C. Chen, D.Z. Liu, M.T. Sheu, H.O. Ho, Novel injectable thermosensitive hydrogels for delivering hyaluronic acid-doxorubicin nanocomplexes to locally treat tumors, Nanomedicine, 10 (2015) 1263-1274.
[304] G. Bajaj, M.R. Kim, S.I. Mohammed, Y. Yeo, Hyaluronic acid-based hydrogel for regional delivery of paclitaxel to intraperitoneal tumors, J. Control. Release, 158 (2012) 386-392.
[305] G.C. Lee, S.Y. Chae, J.H. Park, Y.J. Kim, Sustained release system of PEG-TRAIL/hyaluronic acid nanocomposite hydrogel for treating and preventing cancer and autoimmune disease,KR2009118629A, 2009
[306] H.J. Byeon, S.H. Choi, J.S. Choi, I. Kim, B.S. Shin, E.S. Lee, E.S. Park, K.C. Lee, Y.S. Youn, Four-arm PEG cross-linked hyaluronic acid hydrogels containing PEGylated apoptotic TRAIL protein for treating pancreatic cancer, Acta Biomater., 10 (2014) 142-150.
[307] K. Xu, F. Lee, S.J. Gao, J.E. Chung, H. Yano, M. Kurisawa, Injectable hyaluronic acid-tyramine hydrogels incorporating interferon-α2a for liver cancer therapy, J. Controlled Release, 166 (2013) 203-210.
[308] E. Montanari, S. Capece, C. Di Meo, M. Meringolo, T. Coviello, E. Agostinelli, P. Matricardi, Hyaluronic acid nanohydrogels as a useful tool for BSAO immobilization in the treatment of melanoma cancer cells, Macromol. Biosci., 13 (2013) 1185-1194.
[309] C. Fu, H. Li, N. Li, X. Miao, M. Xie, W. Du, L.M. Zhang, Conjugating an anticancer drug onto thiolated hyaluronic acid by acid liable hydrazone linkage for its gelation and dual stimuli-response release, Carbohydr. Polym., 128 (2015) 163-170.
[310] O.P. Varghese, W. Sun, J. Hilborn, D.A. Ossipov, In situ cross-linkable high molecular weight hyaluronan-bisphosphonate conjugate for localized delivery and cell-specific targeting: a hydrogel linked prodrug approach, J. Am. Chem. Soc., 131 (2009) 8781-8783.
[311] Y. Lei, S. Huang, P. Sharif-Kashani, Y. Chen, P. Kavehpour, T. Segura, Incorporation of active DNA/cationic polymer polyplexes into hydrogel scaffolds, Biomaterials, 31 (2010) 9106-9116.
[312] Y. Lei, M. Rahim, Q. Ng, T. Segura, Hyaluronic acid and fibrin hydrogels with concentrated DNA/PEI polyplexes for local gene delivery, J. Control. Release, 153 (2011) 255-261.
[313] T.J. Brown, A composition and method for the enhancement of the efficacy of drugs,WO0041730A1, 2000
[314] T.J. Brown, The development of hyaluronan as a drug transporter and excipient for chemotherapeutic drugs, Curr. Pharm. Biotechnol., 9 (2008) 253-260.
[315] P. Gibbs, P.R. Clingan, V. Ganju, A.H. Strickland, S.S. Wong, N.C. Tebbutt, C.R. Underhill, R.M. Fox, S.P. Clavant, J. Leung, M. Pho, T.J. Brown, Hyaluronan-Irinotecan improves progression-free survival in 5-fluorouracil refractory patients with metastatic colorectal cancer: A randomized phase II trial, Cancer Chemother. Pharmacol., 67 (2011) 153-163.
[316] HA-Irinotecan in patients with metastatic colorectal cancer, Alchemia web site, 2015.
[317] P. Gibbs, T.J. Brown, R. Ng, R. Jennens, E. Cinc, M. Pho, M. Michael, R.M. Fox, A pilot human evaluation of a formulation of irinotecan and hyaluronic acid in 5-fluorouracil-refractory metastatic colorectal cancer patients, Chemotherapy, 55 (2009) 49-59.
[318] H. Riechelmann, A. Sauter, W. Golze, G. Hanft, C. Schroen, K. Hoermann, T. Erhardt, S. Gronau, Phase I trial with the CD44v6-targeting immunoconjugate bivatuzumab mertansine in head and neck squamous cell carcinoma, Oral Oncol., 44 823-829.
[319] K.Y. Choi, K.H. Min, H.Y. Yoon, K. Kim, J.H. Park, I.C. Kwon, K. Choi, S.Y. Jeong, PEGylation of hyaluronic acid nanoparticles improves tumor targetability in vivo, Biomaterials, 32 (2011) 1880-1889.
[320] A.D. Wong, M. Ye, M.B. Ulmschneider, P.C. Searson, Quantitative analysis of the Enhanced Permeation and Retention (EPR) effect, PLoS One, 10 (2015).

[321] G.J.R. Charrois, T.M. Allen, Multiple Injections of Pegylated Liposomal Doxorubicin: Pharmacokinetics and Therapeutic Activity, J. Pharmacol. Exp. Ther., 306 (2003) 1058-1067.