Liposomal nanomedicines in the treatment of prostate cancer

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A B S T R A C T

Prostate cancer is the most common cancer type and the second leading cause of death from cancer in males. In most cases, no curative treatment options are available for metastatic castration-resistant prostate cancer as these tumors are highly resistant to chemotherapy. Targeted drug delivery, using liposomal drug delivery systems, is an attractive approach to enhance the efficacy of anticancer drugs and prevent side effects, thereby potentially increasing the therapeutic index. In most preclinical prostate cancer studies, passive liposomal targeting of anticancer drugs (caused by enhanced permeability and retention of the therapeutic compound) leads to an increased antitumor efficacy and decreased side effects compared to non-targeted drugs. As a result, the total effective dose of anticancer drugs can be substantially decreased. Active (ligand-mediated) liposomal targeting of tumor cells and/or tumor-associated stromal cells display beneficial effects, but only limited preclinical studies were reported. To date, clinical studies in prostate carcinoma have been performed with liposomal doxorubicin only. These studies showed that long-circulating, PEGylated, liposomal doxorubicin generally outperforms conventional short-circulating liposomal doxorubicin, stressing the importance of passive tumor targeting for this drug in prostate carcinoma. In this review, we provide an overview of the (pre)clinical studies that focus on liposomal drug delivery in prostate carcinoma.

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Introduction on prostate cancer and liposomal drug delivery

Over the past decades, substantial progress has been made in the field of nanomedicinal drug delivery [1,2]. In this booming field, liposomes have taken a front-runner position and have been evaluated extensively in preclinical and clinical cancer settings. Meanwhile, a few liposomal formulations have been clinically approved for the treatment of cancer [3]. Among the extensive amount of studies in the field of liposomal tumor targeting, only a limited number of investigations have focused on the utility of liposomes in prostate cancer treatment. It is striking that amongst those studies, castration-resistant prostate cancer (CRPC) has deserved relatively little attention, as CRPC is one of the most detrimental among the advanced-stage cancers, with very little effective treatment options currently available. Many drugs designed for the treatment of CRPC fail at some point during clinical development due to intrinsic/acquired resistance and/or dose-limiting side effects. Described mechanisms for therapy resistance include overexpression of P-glycoprotein [4] and enhanced STAT1 expression [5]. Targeted drug delivery systems like liposomes may help overcome drug resistance as higher drug levels are potentially achievable at the tumor site. In addition, targeted drug delivery can diminish drug exposure of healthy tissues leading to less systemic side effects. In light of the extensive experience with several liposomal anticancer formulations [6], liposomal targeting of anticancer drugs to tumors in patients with prostate cancer seems a plausible drug targeting approach.

Liposomes are versatile, self-assembling, carrier materials that contain one or more lipid bilayers, and can be used to encapsulate hydrophilic drugs in their inner aqueous compartment(s) while more hydrophobic drugs can associate with the lipid bilayer(s) (Fig. 1) [reviewed in [7,8]]. Compared to other nanocarriers, liposomes are relatively easy to prepare, biodegradable and essentially...
tered long-circulating liposomes (Fig. 2, upper left) [7,8]. Con-
represents the major targeting principle for intravenously adminis-
tor localization process is referred to as 'passive targeting' and
targeting ligands are used to interact with the tumor target site, this
enhanced permeability and retention (EPR) effect. Because no specific
Hence, this tumor targeting mechanism is referred to as the en-

efficacy of liposome-encapsulated anticancer agents[15].
and subsequent cellular internalization determine the therapeutic
endocytosis can take place. Both the extent of tumor localization
binding to the receptor, internalization via receptor-mediated

display a chaotic and highly permeable vasculature as a result of
angiogenesis, the long circulation time of PEG-liposomes allows en-
hanced extravasation of liposomes into the tumor microenviron-
ment compared to healthy tissues. Generally, an increased
liposomal size favors extravasation as long as this size does not ex-
ceed the size of the inter-endothelial fenestrae, which are typically
200–400 nm [11–13]. After extravasation, liposomes are usually re-
tained since lymphatic drainage is often impaired in tumors [7].
Hence, this tumor targeting mechanism is referred to as the en-
hanced permeability and retention (EPR) effect. Because no specific
targeting ligands are used to interact with the tumor target site, this
tumor localization process is referred to as ‘passive targeting’ and
represents the major targeting principle for intravenously adminis-
tered long-circulating liposomes (Fig. 2, upper left) [7,8]. Con-
versely, ‘active targeting’ implies a ligand or antibody bound to the
outer surface of liposomes that selectively target receptors/ligands
overexpressed on the tumor cells (Fig. 2, upper right) or the (a)cel-

ternal tumor microenvironment (Fig. 2, lower left) [7,8,14]. Following
binding to the receptor, internalization via receptor-mediated
endocytosis can take place. Both the extent of tumor localization
and subsequent cellular internalization determine the therapeutic
efficacy of liposome-encapsulated anticancer agents [15].

The aim of this review is to summarize the literature on both
passively and actively targeted liposomes for the treatment of
prostate cancer, and to provide a perspective on the use of targeted
liposomes as a new therapeutic option to treat this malignancy.

Preclinical studies
A limited number of studies focused on passive and/or active
liposomal targeting of chemotherapeutic agents in preclinical
prostate cancer models. Chemotherapy is widely used to treat
prostate carcinoma, but is reserved only for the later stages of
the disease, when the disease has progressed into the stage of CRPC
for which typically a combination of docetaxel and prednisone is
given [16,17]. Unfortunately, only a small proportion of patients
respond to docetaxel and dose-limiting myelosuppression prohib-
its intensification of treatment [16]. This unfavorable situation pro-
vides a strong rationale for tumor-targeted delivery of chemotherapeutic agents.

A phase I study with liposomal docetaxel was conducted in a
cohort of multiple advanced solid malignancies which revealed
higher maximum tolerated dosages of the liposomal formulation
compared to free docetaxel (85 mg/m², or 110 mg/m² with G-SCF
support; compared to 75 mg/m² for free docetaxel) [18]. Surpris-
ingly, while being the standard-of-care for CRPC, liposomal doce-
taxel has not yet been investigated in preclinical models of
prostate cancer. This is even more striking considering the range of
studies that have been performed with liposomal formulations of
other chemotherapeutic agents, including doxorubicin [19–23],
gemcitabine [24,25], paclitaxel [26] and mitoxantrone [27].

Doxorubicin, an anthracycline widely used as chemotherapeu-
tic agent, is associated with several side effects, most notably card-
dioxicity [28], and liposomal delivery of doxorubicin was proven
useful to reduce chronic cardiotoxicity. As a result, liposomal deliv-
ery increases the therapeutic index of the drug. Indeed, liposomal
doxorubicin has been clinically approved for the treatment of
Kaposi’s sarcoma, ovarian cancer, breast cancer and multiple mye-
loma (as PEG-liposomal doxorubicin marketed as Doxil in the USA
and Caelyx outside the USA) and for advanced breast cancer (the
non-PEGylated liposomal doxorubicin version marketed as Myo-
cet) [3,29].

Passive delivery of liposomal doxorubicin was examined in
multiple human prostate cancer cell line-based and primary pros-
tate cancer-based in vivo models. Monotherapy with liposomal
doxorubicin resulted in contrasting results, with three studies
showing significant inhibition of subcutaneous tumor growth
[19–21] while one study showed no effect [22]. It is hard to pin-
point the reason for these differential responses, as there were dif-
ferences in liposomal compositions, size, tumor models, dosing and
time of treatment. Liposomal delivery of gemcitabine, a nucleoside
analog clinically used for several types of cancer, induced a potent
antitumor effect which could only be matched by 45-fold higher
doses of free gemcitabine (8 mg/kg/week versus 360 mg/kg/week,
respectively) [24,25]. Moreover, decreased numbers of lymph node
metastases were observed upon treatment with liposomal gemcit-
abine compared to free gemcitabine [25]. Liposomal delivery of
mitoxantrone, the previous second-line treatment for CRPC,
showed an inhibition of prostate xenograft growth but was not com-
pared to free mitoxantrone [27].

In contrast to doxorubicin and gemcitabine, liposomal delivery
of paclitaxel does not lead to a better outcome, as was evidenced
by a study in a rat prostate cancer xenograft model. Here, efficient
tumor inhibition by liposomal paclitaxel was observed at the cost
of severe weight loss [26], indicative of excessive systemic toxicity.
It may therefore be doubtful whether or not liposomal delivery will
increase the therapeutic index of paclitaxel in advanced prostate
cancer.

In the attempts to further enhance the efficacy of liposomal
anticancer drug targeting, two approaches deserve attention: com-
bination therapy and active targeting. Combination therapy of li-
osomal doxorubicin with radiation [19] or low frequency ultrasound
[22] enhanced the antitumor efficacy compared to liposomal doxo-
rubicin alone. In addition, ultrasound was shown to enhance the
penetration of released doxorubicin throughout the prostate xe-
ograft, thereby also reaching tumor cells further removed from the
blood vessels [23].
Active targeting of receptors on tumor cells and on cancer-associated cells has been pursued, as both tumor and stromal cells may have distinct cellular characteristics which enable selective targeting. In a prostate cancer xenograft model, active targeting of liposomal doxorubicin with an anasamide-PEG derivate to sigma receptors (overexpressed in prostate cancer-derived cell lines) displayed improved antitumor efficacy compared to passively targeted doxorubicin, while free doxorubicin treatment was associated with severe systemic toxicity and treatment-related death [20]. Another active targeting approach focused on fibroblast growth factor receptors (FGFRs), frequently overexpressed in tumor cells and tumor-associated vasculature. In a TRAMP-C1 xenograft model, active FGF-based liposomal delivery of doxorubicin led to a massive reduction in tumor growth and prolonged survival when compared with passively targeted doxorubicin and free doxorubicin [21]. It is unclear to what extent the enhanced antitumor effects were mediated by direct (tumor), indirect (supportive stroma) or combined effects on FGFR-expressing cells. Furthermore, active targeting of tumor vasculature with asparagine-glycine-arginine-(NGR)-targeted liposomes has been explored for prostate carcinoma. NGR selectively binds a tumor endothelium-specific CD13 isoform and displays a high binding capacity to cultured human vascular endothelial cells (HUVEC) in vitro. Active, NGR-based targeting of doxorubicin induced a dose-dependent inhibition of prostate tumor growth (1–6 mg/kg/week) but was not compared to passively targeted liposomes [31].

In addition to chemotherapeutics, bisphosphonates provide a group of antiresorptive drugs clinically relevant for the treatment of prostate cancer patients with metastatic bone disease. Bisphosphonates home to bone very efficiently due to high affinity for hydroxyapatite which is abundantly present in the calcified bone matrix. At this site, osteoclastic bone resorption is inhibited and for this reason bisphosphonates are widely used in the clinic to prevent tumor-induced bone loss [32]. Several studies highlight the depleting effect of free and liposomal bisphosphonates on tumor-associated macrophages (TAMs) from the tumor microenvironment [33–35]. TAMs are involved in tumor-associated inflammation by secretion of a wide range of cytokines and other inflammatory factors including VEGF, EGF and MMP-9, and they contribute to tumor progression, invasion and angiogenesis [32]. Thus, liposomal targeting of bisphosphonates provides a potential approach to dampen tumor-associated inflammation and tumor progression. Indeed, intravenous injection of liposomal bisphosphonate zoledronic acid resulted in decreased levels of TAMs, reduced angiogenesis and inhibition of prostate xenograft growth [36,37]. In metastatic xenograft models, liposomal delivery of another bisphosphonate, clodronate, led to inhibited metastatic growth and reduced numbers of bone metastases which were accounted to a reduction in TAMs [38,39], reduced levels of inflammatory cytokine IL-6 [39] and a reduction of osteoclast activity [39].

Besides therapeutic potential of bisphosphonates, they can also be used as active targeting devices to selectively deliver anticancer Fig. 2. Enhanced permeability and retention (EPR) effect and different modes of liposomal drug delivery. Tumors often display a chaotic and highly permeable vasculature as a result of angiogenic and vascular permeability factors (e.g. VEGF). The long circulation time of PEG-liposomes allows enhanced extravasation of liposomes into the tumor microenvironment. In addition, the lack of proper lymphatic drainage system further contributes to the EPR effect. This so-called passive targeting represents a major targeting principle for liposomes (upper left panel). Active targeting involves a ligand bound to the outer surface of liposomes that selectively target receptors/ligands overexpressed on the tumor cells (upper right panel) or the (a)cellular tumor microenvironment (lower left panel). Following binding to the receptor, internalization via receptor-mediated endocytosis can take place.
drugs to bone metastases. As mentioned earlier, hydroxyapatite is abundantly exposed in the microenvironment of bone metastases leading to enhanced binding by bisphosphonate structures. Indeed, liposomes with a bisphosphonate-moiety display efficient binding to hydroxyapatite in vitro [40–42]. However, hydroxyapatite binding was decreased at increasing serum levels, pointing to competition between serum proteins and bisphosphonate-decorated liposomes [42]. Despite serum competition, it was recently confirmed that bisphosphonate-decorated liposomes display in vivo affinity for collagen/hydroxyapatite scaffolds transplanted in rats [42]. These findings indicate that bisphosphonate-decorated liposomes may provide a means to target anticancer drugs to bone, but active delivery of anticancer drugs has not yet been substantiated and the approach warrants further investigation.

Finally, liposomal delivery of antisense oligonucleotides, which inhibit the translation of target messenger RNAs, was evaluated. Antisense oligonucleotides against nucleic acids coding for oncoproteins may block the production of pivotal proteins for tumor growth. Using such an approach, Bcl-2 provides a promising target since it inhibits apoptosis and is associated with therapy resistance [43]. Targeted knockdown of Bcl-2 may lead to apoptosis induction or sensitization in tumor cells. Interestingly, intravenous administration of PEGylated cationic liposomes containing Bcl-2 antisense RNA resulted in a dose-dependent inhibition of prostate cancer xenograft growth [44]. These findings indicate successful knockdown of Bcl-2 in vivo, though the intra-tumoral protein levels of Bcl-2 were not reported [44]. In a similar way, liposomes were used to selectively knock down PKN3, Raf-1 and TMPRSS2/ERG; proteins associated with prostate cancer growth [45–48]. As such, intravenous liposomal administration of siPKN3 led to a significant decrease in tumor size, as well as a strong reduction in the number of affected lymph nodes but, unfortunately, also downregulated PKN3 in healthy tissues [46]. This may point at suboptimal tumor-specificity of the liposomal system. Liposomal delivery of Raf antisense oligonucleotides led to a 50% knockdown of Raf-1 in tumor tissues, which resulted in an enhanced antitumor activity of docetaxel, cisplatin, epirubicin and mitoxantrone on prostate cancer xenografts [48]. This indicates that liposomal siRNA-mediated protein silencing can sensitize prostate xenografts to chemotherapeutics. Another study monitored the effect of liposomal delivery of si-RNA targeted against the TMPRSS2/ERG fusion gene which revealed a significantly inhibition of subcutaneous and intraprostatic xenograft growth while no toxicity was observed [49].

Active targeting of prostate cancer cells to selectively deliver siRNA was explored in a prostate xenograft model, in which potent in vivo knockdown of target Plk-1 was achieved using PSA-responsive, prostate-specific membrane antigen (PSMA)-targeted liposomes, subsequently leading to decreased tumor growth [50]. These multifunctional liposomes offer enhanced selectivity for prostate cancer cells as both PSA and PSMA should be present to facilitate receptor-mediated endocytosis. As can be deducted from the examples above, targeting proteins that are involved in growth and survival of prostate cancer cells may represent a viable treatment approach. In addition, proteins that are involved in the interaction between tumor cells, stromal cells and the extracellular matrix may provide interesting targets. An important protein involved in this communication is αv-integrin. It was shown that intra-tumoral injection of liposome-encapsulated αv-integrin-siRNA resulted in potent in vivo knockdown and consequently hampered intra-osseous growth of prostate tumor cells [51]. However, intra-tumoral injection is less relevant from a clinical perspective since metastases often present themselves at poorly accessible sites. Targeted delivery of αv-integrin-siRNA after systemic administration was not explored in this study [51].

Clinical studies

With promising preclinical research results with liposomal doxorubicin in prostate cancer models, and the clinical approval of PEGylated and non-PEGylated liposomal doxorubicin in other cancer types, it comes as no surprise that clinical trials with liposomal doxorubicin have also been carried out in CRPC. Indeed, several phase I and phase II trials have been performed evaluating treatment with non-PEGylated [52–54] and PEGylated [55–58] liposomal doxorubicin, either as a monotherapy or in combination with docetaxel [59]. In these studies, serum levels of PSA were used as a read out and a decline of at least 50% was classified as a clinical response. In studies focusing on monotherapy with liposomal doxorubicin, it was notable that patients treated with PEGylated liposomal doxorubicin seemed to respond better compared to patients treated with non-PEGylated liposomal doxorubicin. For example, in the study of McMenemin et al., treatment with PEGylated liposomal doxorubicin leads to a clinical response in 4 out of 14 patients with hormone refractory prostate carcinoma and bone metastases. In another study by Flaherty and coworkers no clinical responses were seen in nine hormone refractory prostate cancer patients treated with non-PEGylated liposomal doxorubicin [52]. Taken together, at least 50% reduction in PSA levels was observed in 18/88 patients (20%, range 11–28%) versus 10/77 patients (13%, range 0–15%) upon treatment with PEGylated and non-PEGylated liposomal doxorubicin, respectively. Of note, different dosing regimens were used in different studies, in which infrequent treatment with high-dose PEGylated liposomal doxorubicin (every 3 or 4 weeks) [55–58] resulted in PSA responses while frequent low-dose treatment (every week) did not [56]. This is exemplified by a phase II trial in which 50 mg/m² every 4 weeks led to substantial PSA decreases whereas 25 mg/m² every 2 weeks did not [56]. Even with the most effective dosing schedule of PEGylated doxorubicin, however, only a small proportion of the patient population shows an antitumor response (ranging from 11% to 28%).

As synergistic effects of doxorubicin and docetaxel were described in human prostate cancer cells in vitro [60], combination treatment of liposomal doxorubicin with (non-liposomal) docetaxel was evaluated, which shows ≥50% decline in PSA level in 50% of the patients with prostate cancer [59]. Treatment with docetaxel alone, however, already results in a PSA decline in 45–48% of the patients [16], so it is doubtful if addition of liposomal doxorubicin adds much value.

In addition to clinical responses, treatment-associated toxicities were monitored. Compared to free doxorubicin, liposomal encapsulation of doxorubicin resulted in reduced cardiotoxicity and less severe myelosuppression, but led to increased dose-limiting skin and mucosal toxicities [55,61] including hand-foot syndrome and stomatitis [55,62,63]. This shift in safety profile for liposomal treatment with non-PEGylated liposomal doxorubicin seemed to respond better compared to patients treated with PEGylated liposomal doxorubicin, either as a monotherapy or in combination with docetaxel [59]. In a phase II trial in which 50 mg/m² every 4 weeks led to substantial PSA decreases whereas 25 mg/m² every 2 weeks did not [56]. Even with the most effective dosing schedule of PEGylated doxorubicin, however, only a small proportion of the patient population shows an antitumor response (ranging from 11% to 28%).

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Conclusions

This review aims to provide a summary of the studies done so far with liposomal drug delivery to prostate carcinoma and its bone metastases. Different potential anticancer drugs were studied including several chemotherapeutics, bisphosphonates and antisense nucleotides (Table 1). In general, passive liposomal targeting of chemotherapeutic doxorubicin displays increased antitumor
Overview of preclinical studies on liposomal drug targeting in prostate carcinoma models.

<table>
<thead>
<tr>
<th>Antitumor drug</th>
<th>Liposome composition</th>
<th>Dose &amp; administration</th>
<th>Mean liposome size</th>
<th>Passive/active targeting</th>
<th>In vivo model: animals, cells, tumor inoculation site</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>DSPE, DSPC, DSPE-PEG2000, CHOL (e.g. Caelyx)</td>
<td>3.5 mg/kg, i.v.</td>
<td>96 nm</td>
<td>Passive</td>
<td>Balb-c nu/nu, human primary cells, s.c.</td>
<td>[19]</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>PC, CHOL, DSPE-PEG-SF2-2AA</td>
<td>7.5 mg/kg/week, i.v.</td>
<td>Not shown</td>
<td>Active, anisamide</td>
<td>Female athymic nude mice, Du145, s.c.</td>
<td>[20]</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>DOTAP, CHOL, tbfGFCF</td>
<td>5 mg/kg/2x week, i.v.</td>
<td>162 nm</td>
<td>Active, tbfGFCF</td>
<td>C57BL/6 J, TRAMP-C1, s.c.</td>
<td>[21]</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>DSPE, DSPC, DSPE-PEG2000, CHOL (e.g. Caelyx)</td>
<td>3.5 mg/kg, i.v.</td>
<td>85 nm</td>
<td>Passive</td>
<td>Balb-c nu/nu, human primary cells, s.c.</td>
<td>[22]</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>DEPC, DSPC, DSPE-PEG, CHOL</td>
<td>16 mg/kg, i.v.</td>
<td>90 nm</td>
<td>Passive</td>
<td>Female Balb-c nu/nu, PC-3, s.c.</td>
<td>[23]</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>PC, DSPE-PEG-NGR, CHOL</td>
<td>1–6 mg/kg/week, i.v.</td>
<td>Not shown</td>
<td>Active, NGR</td>
<td>Male athymic nude mice, PC-3, s.c.</td>
<td>[31]</td>
</tr>
<tr>
<td>Gemcitabine</td>
<td>PC, CHOL, no PEG</td>
<td>6–8 mg/kg/week, i.v.</td>
<td>Not shown</td>
<td>Passive</td>
<td>SCID, Du145/PC-3, s.c.</td>
<td>[24]</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>PC, CHOL, no PEG</td>
<td>8 mg/kg/week, i.v.</td>
<td>36 nm</td>
<td>Passive</td>
<td>SCID, LNCaP, intraprostatic</td>
<td>[25]</td>
</tr>
<tr>
<td>Imatinib- mitoxantrone</td>
<td>DSPC, CHOL, no PEG</td>
<td>5 mg/kg/4 times in 8 days, i.v.</td>
<td>Not shown</td>
<td>Passive</td>
<td>Copenhagen rats, MatLa, s.c.</td>
<td>[26]</td>
</tr>
<tr>
<td>Zoledronic acid</td>
<td>PC, DSPE-PEG2000, CHOL</td>
<td>0.5–2 mg/kg/week, i.v.</td>
<td>Not shown</td>
<td>Passive</td>
<td>Swiss mice nu/nu, PC-3, s.c.</td>
<td>[27]</td>
</tr>
<tr>
<td>Clodronate</td>
<td>PC, CHOL, no PEG</td>
<td>Every 3 days, s.c.</td>
<td>Not shown</td>
<td>Passive</td>
<td>Balb-c nu/nu, HARA-B, intracardiac</td>
<td>[38]</td>
</tr>
<tr>
<td>Bel-2 siRNA</td>
<td>PC, CHOL, PEG, CLZ</td>
<td>3x every 5 days, i.p.</td>
<td>Not shown</td>
<td>Passive</td>
<td>NCI-nu, PC-3, intrasosseous</td>
<td>[39]</td>
</tr>
<tr>
<td>PKN3 siRNA</td>
<td>DPhyPE, DSPE-PEG</td>
<td>2.8 mg/kg, i.v.</td>
<td>118 nm</td>
<td>Passive</td>
<td>NMRI nu/nu, PC-3, s.c.</td>
<td>[46]</td>
</tr>
<tr>
<td>Raf antisense</td>
<td>DDAB, PC, CHOL, no PEG</td>
<td>l.v.</td>
<td>Not shown</td>
<td>Passive</td>
<td>Male athymic nu/nu, PC-3, s.c.</td>
<td>[47–48]</td>
</tr>
<tr>
<td>TMRPSS2/ERG siRNA</td>
<td>DOTAP, DOPC</td>
<td>Twice weekly, 150 μg/kg, i.v.</td>
<td>65 nm</td>
<td>Passive</td>
<td>SCID, VCaP, s.c., intraprostatic</td>
<td>[49]</td>
</tr>
<tr>
<td>PLK-1 siRNA</td>
<td>SPC, DSPE-PEG2000, DSPE-PEG2000-ACPP, DSPE-PEG5000-Folate, CHOL-DC-CHOL</td>
<td>1.5 mg/kg/ever 2 days, i.v.</td>
<td>208 nm</td>
<td>Active, Folate</td>
<td>Balb-c nu/nu, 22Rv1, s.c.</td>
<td>[50]</td>
</tr>
<tr>
<td>9v-Integrin siRNA</td>
<td>DPPC, DPE, DPPE, PEG</td>
<td>1 μg, i.t.</td>
<td>Not shown</td>
<td>Passive</td>
<td>Balb-c nu/nu, PC-3, intrasosseous/s.c.</td>
<td>[51]</td>
</tr>
</tbody>
</table>

**Abbreviations:** siRNA, small interfering RNA; i.v., intravenous; s.c., subcutaneous; i.t., intratumoral; CHOL = cholesterol; PC = phosphocholine; DOTAP = dioleoyl trimethylammonium propane; DDAB = dimethyldioctadecylammoniumbromide; SPC = soybean phosphatidylcholine; DOPC = 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine.

efficacy compared to free doxorubicin, as well as a decrease of side effects. This antitumor effect can potentially be further enhanced by combination therapy or active targeting. Here, targeting of both tumor and stromal cells offers a valuable approach, as exemplified by FGFR-targeted doxorubicin liposomes that show a highly potent antitumor response in a preclinical study.

Amongst the performed passive targeting studies, liposomal delivery of gemcitabine seems highly promising as it allows a massive dose reduction while yielding similar antitumor effects as a 45-fold higher free gemcitabine dosage. Liposomal gemcitabine almost completely arrests prostate cancer xenograft growth whereas liposomal doxorubicin only seems to slow it down. Based on this, further investigation on targeted delivery of gemcitabine in preclinical prostate cancer models is warranted.

It is important to note, though, that most studies use subcutaneous tumor models to monitor prostate cancer growth in vivo. Although these studies can be useful, the models may not be representative of drug delivery to the relevant biological site, e.g. (bone) metastatic tumor, as tumor microenvironment, vascularization and other properties may differ between subcutaneous and orthotopically implanted tumors. Hence, the use of more clinically relevant in vivo models is key, specifically models that mimic metastatic tumor growth (especially in the bone), as they represent the clinical stage of advanced, incurable, prostate cancer. Indeed, intrabone and intracardiac tumor inoculation, which appear most useful in this regard, were sporadically used in literature as models for experimentally-induced prostate cancer metastases to evaluate liposomal targeting approaches.

The bone/bone-marrow microenvironment, the main site of prostate cancer metastasis, has unique features that can be exploited to selectively deliver anticancer drugs to bone metastases. As such, bisphosphonate-coated liposomes provide a promising, yet underexplored, platform with effective targeting affinity for active bone surfaces (hydroxyapatite), which is abundantly exposed in the local bone metastatic environment. At this site, tumor-associated macrophages promote tumor growth by direct release of growth and inflammatory factors (EGF, VEGF, IL-10, IL-12, TNF-α, amongst others) [66,65] while osteoclasts mediate bone resorption, which leads to the release of bone-matrix bound growth factors (for example, TGF-β) [66]. In a broader perspective, we believe that targeting the supportive bone stroma has clear therapeutic potential, as exemplified by the preclinical studies that show that depletion of tumor-associated macrophages by liposomal delivery of bisphosphonates effectively inhibits bone metastatic growth.

Although preclinical studies with a range of anticancer drugs show the potential of liposomal drug delivery in prostate cancer treatment, passive targeting studies were only performed with four chemotherapeutic agents so far while only one (doxorubicin) was evaluated in an active targeting approach. Also, despite these and other promising preclinical studies with a range of anticancer drugs, only liposomal doxorubicin has been tested in phase I and II
trials for the treatment of prostate cancer (Table 2). Hence, we believe that many challenges remain in the field of liposomal drug delivery for prostate cancer. For the development of such targeting approaches, it is of pivotal importance to exploit prostate tumor and tumor environment specific characteristics which will likely result in the identification of numerous potential new targets, for which novel liposomal anticancer drugs can be designed and developed. Ultimately, this may lead to the development of highly efficient and specific liposomal drug carriers for the treatment of advanced prostate cancer.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgements

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References


Table 2


<table>
<thead>
<tr>
<th>Therapeutic</th>
<th>PEGylated?</th>
<th>Dose intensity (mg/m²)</th>
<th>Regimen</th>
<th>PSA &gt; 50% decline</th>
<th>Toxicities</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposomal doxorubicin</td>
<td>No</td>
<td>10–20</td>
<td>10–20 mg/m² weekly for 4 weeks out of a 6-week cycle</td>
<td>0/9 (9%)</td>
<td>No Grade III or IV</td>
<td>[52]</td>
</tr>
<tr>
<td>Liposomal doxorubicin</td>
<td>No</td>
<td>12.5 mg/m²</td>
<td>50 mg/m² every 4 weeks</td>
<td>2/14 (14%)</td>
<td>Grade III neuropenia, mucositis and hand-foot syndrome</td>
<td>[53]</td>
</tr>
<tr>
<td>Liposomal doxorubicin</td>
<td>No</td>
<td>25 mg/m²</td>
<td>25 mg/m² weekly</td>
<td>8/54 (15%)</td>
<td>Grade III neuropenia</td>
<td>[54]</td>
</tr>
<tr>
<td>Liposomal doxorubicin</td>
<td>Yes</td>
<td>15 mg/m²</td>
<td>45 mg/m² every 3 weeks or 60 mg/m² every 4 weeks</td>
<td>3/15 (20%)</td>
<td>Grade III/IV stomatitis and hand-foot syndrome</td>
<td>[55]</td>
</tr>
<tr>
<td>Liposomal doxorubicin</td>
<td>Yes</td>
<td>12.5 mg/m²</td>
<td>23 mg/m² every 2 weeks or 50 mg/m² every 4 weeks</td>
<td>8/31 (26%)</td>
<td>Grade III/IV tachycardia, hepatic toxicity and hemoglobin toxicity</td>
<td>[56]</td>
</tr>
<tr>
<td>Liposomal doxorubicin</td>
<td>Yes</td>
<td>12.5 mg/m²</td>
<td>50 mg/m² every 4 weeks</td>
<td>4/14 (28%)</td>
<td>Grade III/IV neuropenia, mucositis and skin toxicity</td>
<td>[57]</td>
</tr>
<tr>
<td>Liposomal doxorubicin, docetaxel</td>
<td>Yes</td>
<td>10 mg/m²</td>
<td>6–16 mg/m², 20–35 mg/m²</td>
<td>6–16 mg/m², 20–35 mg/m² every 3 weeks out of a 4-week cycle</td>
<td>3/28 (11%)</td>
<td>Grade III/IV thrombocytopenia</td>
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<tr>
<td>Liposomal doxorubicin, docetaxel</td>
<td>Yes</td>
<td>25 mg/m²</td>
<td>25 mg/m² weekly</td>
<td>12/24 (50%)</td>
<td>Grade III/IV neutropenia, mucositis and asepsis</td>
<td>[59]</td>
</tr>
<tr>
<td>Liposomal doxorubicin, docetaxel</td>
<td>Yes</td>
<td>60 mg/m² every 4 weeks</td>
<td>4/14 (28%)</td>
<td>Grade III/IV mucositis</td>
<td>[52]</td>
<td></td>
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</table>


