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Meta-analysis of clinical and preclinical studies comparing the anticancer efficacy of liposomal versus conventional non-liposomal doxorubicin☆,☆☆

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ABSTRACT

While liposome-mediated delivery of cytotoxic chemotherapy has been shown to significantly enhance drug tolerability in patients as compared to the conventional formulation, the fundamental question remains whether they also improve anticancer efficacy. Thus, we performed a systematic literature search for randomized clinical trials directly comparing efficacy of liposomal cytotoxic chemotherapy versus their equivalent conventional formulation. The search yielded 14 clinical trials (8 anthracycline, 4 cisplatin, 1 paclitaxel, 1 irinotecan) that meet inclusion criteria, with a total of 2589 patients. We found that efficacy in patients was not different between liposomal and conventional chemotherapy as assessed by objective response (odds ratio 1.03; 95% confidence interval [CI] 0.82–1.30), overall survival (hazard ratio [HR] 1.05; 95% CI 0.95–1.17), and progression free survival rates (HR 1.01; 95% CI, 0.92–1.11). Subgroup analyses of only the anthracycline trials also did not show any efficacy advantage for the liposomal formulation. Since pegylated liposomal doxorubicin (PLD) was the most prevalent formulation in these clinical trials, we also performed a meta-analysis of 11 preclinical studies comparing efficacy of PLD and conventional doxorubicin in tumor-bearing mice. In contrast with clinical results, animal studies showed significantly increased survival in mice treated with PLD compared to conventional doxorubicin (HR 0.39; 95% CI 0.27–0.56). We discuss the possible reasons why the pharmacological advantages of carrier-mediated chemotherapy did not translate into enhanced clinical efficacy including the role of the enhanced permeability and retention (EPR) effect and the tumor microenvironment, the optimal dosing regimen for carrier-mediated agents, and the lack of standardization in the conduct and reporting of preclinical studies evaluating anticancer efficacy of these agents. Our study shows that the full clinical potential of carrier-mediated drugs remains to be realized and highlights some of the critical knowledge gaps that must be addressed in order to move the field forward.

1. Introduction

Targeted drug delivery to tumor tissue remains one of the major challenges in the treatment of cancer and systemically administered therapies continue to have severe dose limiting toxicities. Nanoparticle carriers, typically between 10 and 200 nm in size, have tremendous therapeutic potential in the treatment of cancer because they can increase tumor drug delivery while limiting normal tissue distribution and can protect the drug from degradation [1–3]. Theoretically, this would increase anticancer efficacy and decrease systemic toxicity.

Pegylated liposomal doxorubicin (PLD; Doxil/Caelyx) is regarded as the first approved anticancer nanoparticle and has been shown to improve patient tolerability as compared to conventional chemotherapy [4,5]. A major limitation of conventional doxorubicin is cumulative dose-related cardiac toxicity. However cardiac toxicity was not observed with the nanoparticle formulation, PLD, until significantly higher lifetime doses are reached [6–8]. This enhanced tolerability and tumor drug delivery should result in increased anticancer efficacy. However, clinical trials have not demonstrated clear evidence of superior efficacy of PLD over conventional doxorubicin. Over the last two decades, numerous different anticancer nanoparticles have been developed and investigated in hundreds of preclinical and clinical studies. Yet, only a handful has met efficacy criteria for regulatory approval, and the majority of these utilize liposomal platforms (Table 1).

The fundamental question remains whether liposomal formulations of cytotoxic chemotherapies significantly improve anticancer efficacy

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Table 1
Anticancer nanoparticles approved for clinical use.

Trade name	Carrier	Drug cargo	Size	Approved indications
Doxil/Caelyx [9]	PEGylated liposome	Doxorubicin	90 nm	AIDS-related Kaposi sarcoma, multiple myeloma, ovarian cancer
Myocet [10]	Liposome	Doxorubicin	150 nm	Metastatic breast cancer
DaunoXome [11]	Liposome	Daunorubicin	45 nm	AIDS-related Kaposi sarcoma
Marqibo [12]	Liposome	Vincristine	100 nm	Acute lymphoblastic leukemia
Onivyde [13]	PEGylated liposome	Irinotecan	110 nm	Pancreatic adenocarcinoma
DepoCyt [14]	Liposome	Cytarabine	3–30 μm [15]	Lymphomatous meningitis

over conventional non-liposomal formulations. Hence, we performed a meta-analysis of clinical trials comparing the anticancer efficacy of liposomal cytotoxic chemotherapy to their conventional non-liposomal formulations. Moreover, given the low success rates of carrier-mediated drugs in cancer clinical trials, there is an urgent need to understand deficiencies in the current preclinical drug development strategies in order to develop new approaches that are more predictive of clinical results. Since PLD is the most extensively studied liposomal drug, we also performed a meta-analysis of preclinical studies comparing the anticancer efficacy of PLD and conventional doxorubicin in murine tumor models.

2. Methods

2.1. Literature search

A systematic literature search was conducted in PubMed, Scopus, ISI, and Google Scholar for studies published between 1990 and 2015, using the terms “liposom* AND free”, and “liposom* AND conventional”. The article type was filtered to include only clinical trials; no other filters were applied. The full search is available upon request. All the studies and any potentially relevant articles found in their reference lists were reviewed and considered for inclusion.

To identify preclinical studies evaluating liposomal doxorubicin, a systematic literature search was performed similar to methods described above with the following modifications. The search terms used in the databases were “doxorubicin”, “liposomes”, “animals”, “neoplasms OR drug therapy”, and “murine”. No filters were used. As with the clinical literature search, any potentially relevant articles found in reference lists were reviewed and considered for inclusion.

2.2. Inclusion and exclusion criteria

The criteria for inclusion in the meta-analysis of the efficacy of liposomal versus conventional formulations were: randomized trial, contains both a cohort treated with the liposomal formulation and cohort treated with the equivalent conventional formulation, and evaluation of efficacy endpoints (response rate, overall survival, or progression-free survival). All cancer types, pretreatment status, and concurrent treatments were allowed. Studies were excluded if the regimens in the treatment arms differed by more than just liposomal or conventional formulation, efficacy results were not reported, treatment allocation was not randomized, or if the coefficient of variation for data estimated by three independent investigators was >15%.

For the meta-analysis of preclinical studies evaluating liposomal doxorubicin, studies were included if they contained both PLD and conventional doxorubicin treatment cohorts, evaluated anticancer efficacy in at least one tumor model, and published the survival curves. For studies that compared PLD and conventional doxorubicin in more than one tumor model, the survival curve for each tumor model was included. Studies were excluded if the regimens in the treatment arms differed by more than just liposomal or conventional formulation, survival curves were not reported, or if the coefficient of variation for data estimated by three independent investigators was >15%.

2.3. Data extraction

Extraction of study characteristics and efficacy data from text, tables, and figures of included studies was done independently by three investigators. Differences were resolved by consensus. For the clinical trials, study characteristics (first author, journal, year of publication), trial design characteristics (study design, outcome measurement, type of cancer, therapy regimen for each arm), study population (median age, number of patients evaluated for efficacy endpoints in each arm), and efficacy results (hazard ratios [HR] and 95% confidence intervals [CI] for survival data, and objective response rates [ORR]) were recorded. For the preclinical studies, study characteristics (first author, journal, year of publication) and study design characteristics (mouse model, tumor model, number of mice in each cohort, therapy regimen for each cohort, endpoints) were recorded. For all preclinical studies, and for clinical trials in which the HR or a CI for survival data were not provided, three investigators independently analyzed the Kaplan-Meier curves to estimate these data using the method described by Parmar et al. [16]. The coefficient of variation of the data estimated independently by the investigators ranged from 0.85% to 9.86% for the clinical trials and <0.01% to 14.18% for the preclinical studies included in the analyses. The mean of the three independent estimates was used for the meta-analyses. Two preclinical studies were excluded due to a coefficient of variation >15% for their estimated HR, and one preclinical study was excluded due to a 95% CI approaching zero on one side and infinity on the other, indicating a high degree of uncertainty in the estimated HR.

2.4. Statistical analyses

For survival variables such as OS and PFS, the HR and the corresponding 95% CI were used in the meta-analysis with results presented as forest plots. For response rates, the crude OR and the corresponding 95% CI was used in the meta-analysis with results presented as forest plots. Heterogeneity was assessed by Cochran's Q test and I^2 index. A random-effects model (DerSimonian and Laird method) was used for the meta-analysis if the results of the heterogeneity test was $Q > k\text{-}1$, where k is the number of studies, and $P < 0.1$, otherwise a fixed-effect model was used (Mantel-Haenszel method) [17,18]. An overall analysis of all clinical trials and an overall analysis of all preclinical studies were conducted. Subgroup analyses were also carried out as detailed in the subsequent sections. All meta-analyses were performed using MIX 2.0 software (BiostatXL), with a two-tailed P-value of <0.05 considered as statistically significant. Funnel plots with Egger's test were used to detect publication bias [19]. An Egger's regression intercept with a $P < 0.1$ was considered significant for publication bias.

2.5. Assessment of clinical trial quality and design bias

The clinical trial quality and potential design bias were evaluated using the criteria described in the Cochrane handbook for systematic reviews of interventions [20]. These parameters included details of sequence generation, allocation concealment, treatment blinding, completeness of outcomes data, presence of selective outcome reporting, and analysis by intent to treat.

3. Results

3.1. Search results

The search for clinical trials yielded 270 publications, of which 14 met criteria for inclusion in the meta-analysis as summarized in Fig. 1. Of these 14 studies, 8 compared liposomal and conventional anthracycline formulations, 4 compared liposomal and conventional cisplatin formulations, and 1 each compared liposomal and conventional formulations of irinotecan and paclitaxel, respectively. These studies included a total of 2589 patients randomized 1:1 to either liposomal or conventional formulations of chemotherapy who were evaluated for the efficacy endpoints. The characteristics of these studies, including treatment regimens and patient population, are further summarized in Table 2.

For the preclinical studies, the search yielded 224 publications, of which 10 articles containing 11 studies met the criteria for inclusion in the meta-analysis, as summarized in Fig. 2. These studies included a total of 143 mice treated with PLD and 137 mice treated with conventional doxorubicin. The characteristics of these studies, including treatment regimens and tumor models, are summarized in Table 3.

3.2. Meta-analyses

Objective response rates were evaluated in all fourteen trials, and OS and PFS were evaluated in twelve of the fourteen trials. Meta-analysis showed no significant difference between liposomal vs. conventional formulations in objective response rates (OR, 1.03; 95% CI, 0.82–1.30; $p = 0.77$) (Fig. 3), OS (HR, 1.05; 95% CI, 0.95–1.17; $p = 0.34$) (Fig. 4), or PFS (HR, 1.01; 95% CI, 0.92–1.11; $p = 0.86$) (Fig. 5). Subgroup analysis of the eight anthracycline trials also showed no significant difference between liposomal vs. conventional formulations in objective response rates (OR, 0.94; 95% CI, 0.78–1.14; $p = 0.54$), OS (HR, 1.01; 95% CI, 0.89–1.15; $p = 0.84$), and PFS (HR, 1.06; 95% CI, 0.95–1.18; $p = 0.30$). In contrast, subgroup analysis of the four platinum trials showed a significant improvement in objective response rates for liposomal cisplatin compared to conventional cisplatin (OR, 1.49; 95% CI, 1.06–2.09; $p = 0.02$). Subgroup analysis of OS and PFS endpoints were not performed for the cisplatin trials since only 2 of the trials reported these endpoints. Interestingly, the subgroup of platinum trials all comprised non-small cell lung cancer patients suggesting that the moderate improvement in response rate may be related to the drug cargo (i.e., cisplatin) or may be related to the cancer type (i.e., lung cancer). Given that the tumor type specific parameters such as vascularity can impact the pharmacokinetics and pharmacodynamics of liposomal drugs, we also performed a subgroup analysis of only the metastatic breast cancer trials ($n = 3$) which also did not show any improvements in efficacy with the liposomal anthracyclines as compared to conventional formulations (Supplemental Table S1). There were insufficient numbers of trials for subgroup analyses of the other cancer types. Since pegylated and non-pegylated liposomes may have different pharmacokinetic parameters, we performed separate subgroup analyses for pegylated liposomal anthracyclines ($n = 5$) and non-pegylated liposomal anthracyclines ($n = 3$) which also failed to show any efficacy advantages for either of the liposomal formulations (Supplemental Table S1). No evidence of publication bias was detected (Supplemental Fig. S1A-C).

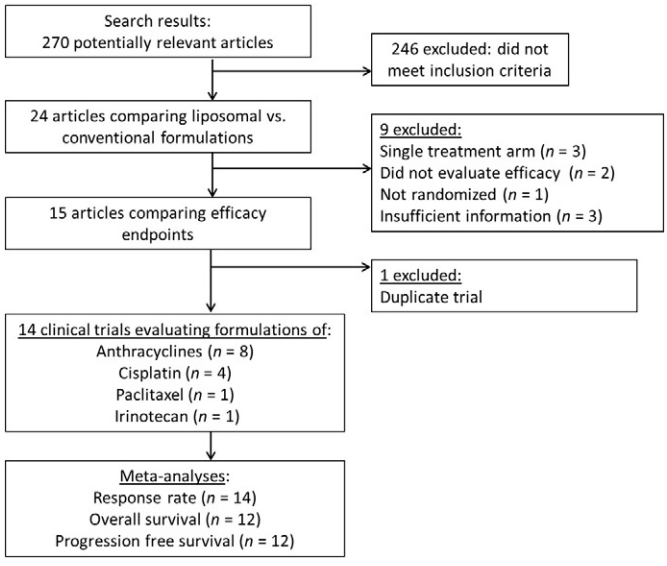

Fig. 1. Flow diagram of the systematic literature search for clinical trials comparing anticancer efficacy of a liposomal formulation of chemotherapy to its conventional non-carrier mediated formulation.

In contrast to the results of the clinical studies, the overall survival rate in mice treated with PLD was significantly higher than in those treated with conventional doxorubicin, HR 0.39 (95% CI 0.27–0.56; $p < 0.0001$) (Fig. 6). The efficacy advantage of the liposomal formulation persists in subgroup analyses of immunocompetent mouse models ($n = 5$) and of mice with severe immunodeficiency ($n = 4$) (Supplemental Table S2). No evidence of publication bias was detected (Supplemental Fig. S1D).

3.3. Clinical trial quality and risk of bias

The potential for study design bias is summarized in Table 4. Although all the clinical trials allocated patients to treatment arms randomly, most did not report how the sequences were generated and whether this was concealed during the trial period. Eight of the fourteen trials were open-label, 5 were conducted with evaluators blinded to treatment assignment, and 1 trial did not publish information on blinding. Intent to treat analysis was reported in 11 of the 14 trials, and only 2 trials showed evidence of incomplete outcomes data. Together, these trial characteristics suggest a moderate risk of study design bias, particularly due to the lack of treatment concealment.

4. Discussion

Our meta-analyses found that the superior antitumor efficacy of liposomal doxorubicin over conventional doxorubicin in preclinical studies generally did not translate to the clinical trial setting. Although these findings are somewhat expected based on the cumulative reports published in the cancer drug delivery field [41], our study is the first to systematically and objectively quantify these effects. The purported advantages of liposomal formulations include longer circulation and enhanced drug delivery to tumor tissue, however, these factors did not lead to enhanced anticancer efficacy in patients. There are many possible explanations for this including the challenges associated with conducting clinical trials and limitations of preclinical tumor models (Fig. 7), and they have been previously reviewed in detail [41]. In our study, most of the patients included in the analyses had advanced or metastatic cancer, were often refractory to multiple treatment modalities, and their malignant diseases typically have arisen and evolved over months and years. In contrast, the murine tumor models in our analyses were predominantly implantable tumors that grow rapidly and reach endpoint within a period of weeks. This rapid growth is often accompanied by high rates of neoangiogenesis which can exaggerate the enhanced permeability and retention (EPR) effect and hence amplify passive tumor targeting by carrier-mediated drugs [42].

Another explanation for the absence of clinical efficacy advantage with the liposomal formulation could be that the dosing regimen may be optimized for the non-liposomal formulation rather than the liposomal formulation. Given the complex and markedly different pharmacokinetics of carrier-mediated drugs as compared to the non-carrier mediated drug [43], it is probable that the optimal regimen for the

Table 2
Characteristics of included clinical studies.

Study (year)	Treatment arms	Number of patients	Median Age (years)	Concurrent treatments	Type of trial	Cancer type
Judson et al. [21]	PLD 50 mg/m^2 every 28 days	50	52	None	phase II	Soft tissue sarcoma
	Doxorubicin 75 mg/m^2 every 21 days	44	52			
Dimopoulos et al. [22]	PLD 40 mg/m^2 every 28 days	132	66	Vincristine Dexamethasone	phase III	Multiple myeloma
	Doxorubicin 9 mg/m^2 every 28 days	127	65			
O'Brien et al. [6]	PLD 50 mg/m^2 every 28 days	254	58	None	phase III	Metastatic breast cancer
	Doxorubicin 60 mg/m^2 every 21 days	255	57			
Rifkin et al. [23]	PLD 40 mg/m^2 every 28 days	97	60	Vincristine Dexamethasone	phase III	Multiple myeloma
	Doxorubicin 9 mg/m^2 every 28 days	95	60			
Hunault-Berger et al. [24]	PLD 40 mg/m^2 days 1–4	31	68	Vincristine Dexamethasone	phase II	Philadelphia chromosome-negative acute lymphoblastic leukemia
	Doxorubicin 12 mg/m^2/day days 1–4	29	66			
Batist et al. [4]	Liposomal doxorubicin 60 mg/m^2 every 21 days	142	55	Cyclophosphamide	phase III	Metastatic breast cancer
	Doxorubicin 60 mg/m^2 every 21 days	155	54			
Harris et al. [25]	Liposomal doxorubicin 75 mg/m^2 every 21 days	108	58	None	phase III	Metastatic breast cancer
	Doxorubicin 75 mg/m^2 every 21 days	116	58			
Latagliata et al. [26]	Daunoxome 80 mg/m^2 days 1–3	148	68	Cytarabine All-*trans* retinoic acid	phase III	Acute myelogenous leukemia
	Daunorubicin 45 mg/m^2 days 1–3	153	68			
Jehn et al. [27]	Liposomal cisplatin 100 mg/m^2 every 21 days	25	56	None	phase III	Squamous cell carcinoma of the head and neck
	Cisplatin 100 mg/m^2 every 21 days	21	58			
Kosmas et al. [28]	Liposomal cisplatin 120 mg/m^2 every 21 days	60	NS	Gemcitabine	phase III	NSCLC
	Cisplatin 100 mg/m2 every 21 days	41	NS			
Mylonakis et al. [29]	Liposomal cisplatin 120 mg/m^2 every 21 days	47	64	Gemcitabine	phase II	NSCLC
	Cisplatin 100 mg/m^2 every 21 days	41	66			
Stathopoulos et al. [30]	Liposomal cisplatin 200 mg/m^2 every 14 days	114	65	Paclitaxel	phase III	Non-squamous NSCLC
	Cisplatin 75 mg/m^2 every 14 days	115	66			
Yang et al. [31]	Liposomal paclitaxel 150 mg/m^2 every 21 days	50	53	Cisplatin	NS	NSCLC
	Paclitaxel 150 mg/m^2 every 21 days	50	55			
Roy et al. [32]	Liposomal irinotecan 120 mg/m2 every 21 days	44	56	None	phase II	gastric or gastro-esophageal adenocarcinoma
	Irinotecan 300 mg/m2 every 21 days	44	62			

PLD, pegylated liposomal doxorubicin; NSCLC, non-small cell lung cancer; NS, not specified.

liposomal formulation will be different than the conventional formulation. The available evidence with liposomal doxorubicin would suggest that there are dose and cycle dependent pharmacokinetic changes that need to be taken into consideration [44] and that larger single doses may be more efficacious than smaller split doses [35]. However, the ideal administration schedule for liposomal drugs remains to be thoroughly evaluated in patients.

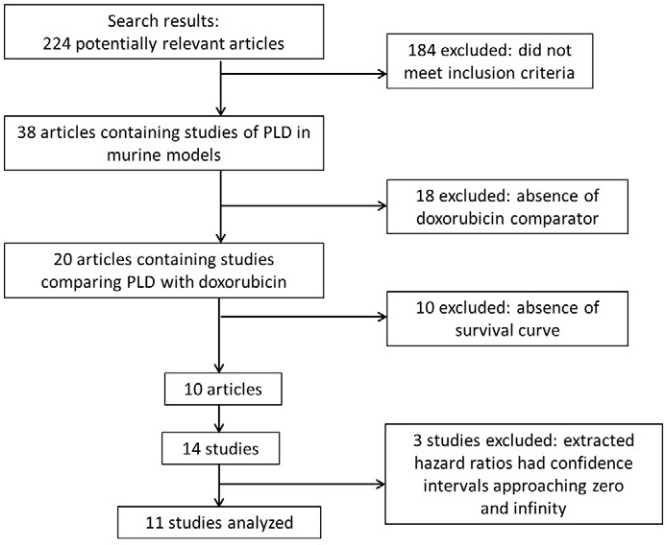

Fig. 2. Flow diagram of the systematic literature search for preclinical studies comparing the anticancer efficacy of liposomal doxorubicin to conventional doxorubicin in mouse models of cancer.

One intriguing explanation for the lack of a clinical efficacy advantage for the liposomal formulation could be the tumor immunologic milieu. The tumor microenvironment has been reported to be infiltrated with immunosuppressive leukocytes such as M2-polarized tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSC), and regulatory T cells that are believed to be key players in the progression of cancer [45–47]. Although nanoparticles are known to be internalized by splenic and hepatic phagocytes, and circulating monocyte numbers and function have been shown to correlate with nanoparticle clearance in patients [48,49], the interactions between nanoparticles and TAMs is relatively unexplored. Recent studies have found that non-drug loaded nanoparticles, including liposomes similar to the PLD carrier, enhanced tumor growth compared to vehicle control in tumor-bearing immunocompetent mice [50,51]. This was associated with polarization of TAMs toward an M2-like phenotype, suppression of antitumor T cell responses, and enhanced tumor angiogenesis in the liposome treated animals [50]. Given this evidence, it is possible that something similar occurs in patients treated with liposomes, such that the benefits of improved drug delivery to tumors are offset by a shift of immune cells toward the tumor-promoting phenotype (Fig. 8).

This effect of the carrier would likely not be detected in our meta-analysis since 5 of the 11 preclinical studies used immunodeficient athymic nude (Nu/nu) mice which lack functional T cells. Moreover, even "wild-type" mouse strains such as DBA/2 can have subtle but observable immune defects due to inbreeding. The DBA/2 mouse strain used in two of the analyzed studies is known to have a loss-of-function mutation in complement component 5 (C5) [52]. Importantly,

Table 3
Characteristics of included preclinical studies.

Study ID	Tumor model (implant route)	Cancer type	Mouse	PLD (n)	Conventional doxorubicin (n)	Other cohorts (n)
[33]	MDA-MB-231-BR (intracranial)	human breast adenocarcinoma	Nu/nu	20	20	PBS (33) ABT-888 (18) dox + ABT-888 (36) PLD + ABT-888 (35)
[34] (A)	M109 (i.p.)	murine lung carcinoma	BALB/c	16	15	No treatment (9) PLD + IL-2 (16) PLD + PEG-SUV-IL-2 (8) PLD + MLV-IL-2 (16) IL-2 (9) MLV-IL-2 (9)
[34] (B)	M109 (i.v.)	murine lung carcinoma	BALB/c	16	16	No treatment (16) PLD + IL-2 (8) PLD + PEG-SUV-IL-2 (15) PLD + MLV-IL-2 (8) IL-2 (8) PEG-SUV-IL-2 (8)
[35]	C26 (s.c.)	murine colon carcinoma	BALB/c	11*	10	No treatment (10)
[36]	C26 (s.c.)	murine colon carcinoma	BALB/c	28	24	Saline (23) Non-PEG L-Dox (29)
[37]	L1210JF (i.p.)	murine leukemia	DBA/2	8	8	Saline (8) Folate-coated PLD (8)
[38]	HTLA-230 (i.v.)	human neuroblastoma	Nu/nu	8*	8	HEPES buffer (8) Free-aGD2 Fab' (8) Fab'-SIL (8) Fab'-SIL + dox (8)
[39] (A)	GI-LI-N (adrenal gland)	human neuroblastoma	Nu/nu	10	10	HBSS (10) TVT-dox (10)
[39] (B)	Colo-699 N (intrapulmonary)	human lung carcinoma	Nu/nu	10	10	HBSS (10) TVT-dox (10)
[39] (C)	OVCAR-3 (i.p.)	human ovarian adenocarcinoma	Nu/nu	10	10	HBSS (10) TVT-dox (10)
[40]	L1210 (s.c.)	murine leukemia	DBA/2	6	6	Cationic L-Dox (6) Neutral L-Dox (6) Empty cationic liposomes (6)

PLD; pegylated liposomal doxorubicin; i.p., intraperitoneal; i.v., intravenous; s.c., subcutaneous; PBS, phosphate buffered saline; ABT-888, A poly-ADP-ribose polymerase (PARP) inhibitor; dox; doxorubicin; PEG-SUV-IL-2, pegylated liposomal interleukin-2; MLV-IL-2, liposomal interleukin-2; Free-aGD2 Fab', Fab' fragment of anti-disialoganglioside antibodies; Fab'-SIL, Fab' fragment of anti-disialoganglioside antibodies coupled to stealth immunoliposomes; HBSS, Hank's balanced salt solution; TVT-dox, tumor vasculature-targeted liposomal doxorubicin; *L*-Dox, liposomal doxorubicin.

* Used the same formulations at multiple dosing schedules; only the most effective regimen of each formulation was included in the analysis.

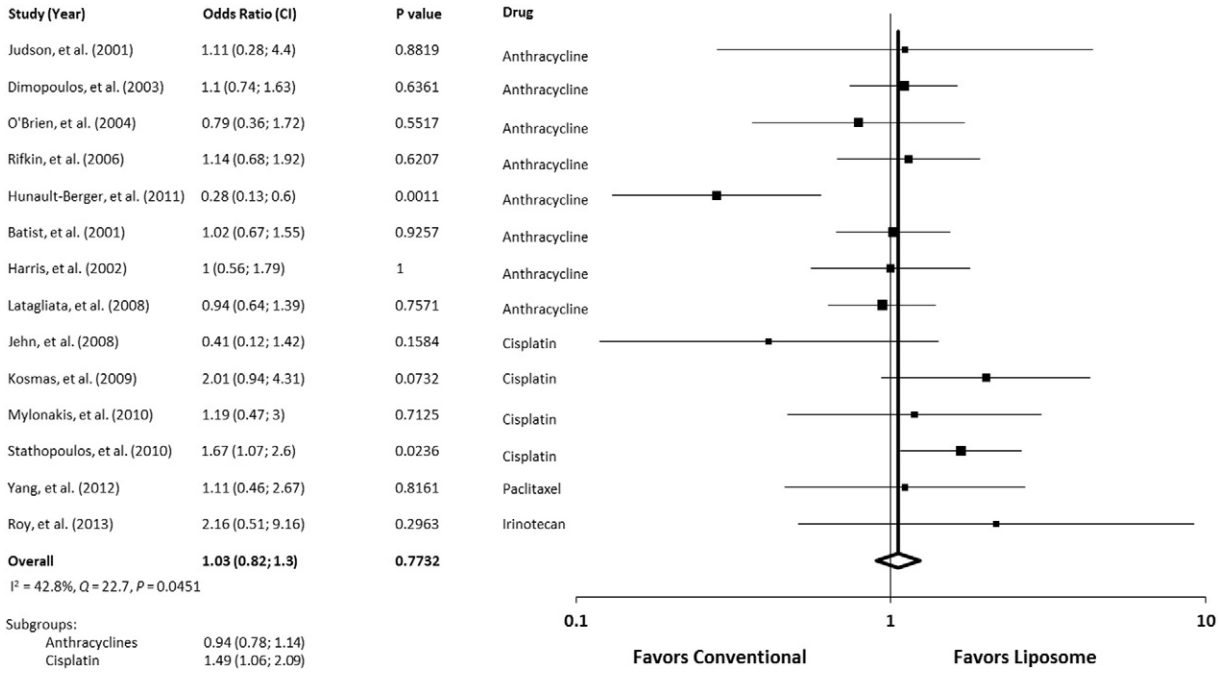

Fig. 3. Objective response in patients treated with liposomal versus conventional chemotherapy formulations.

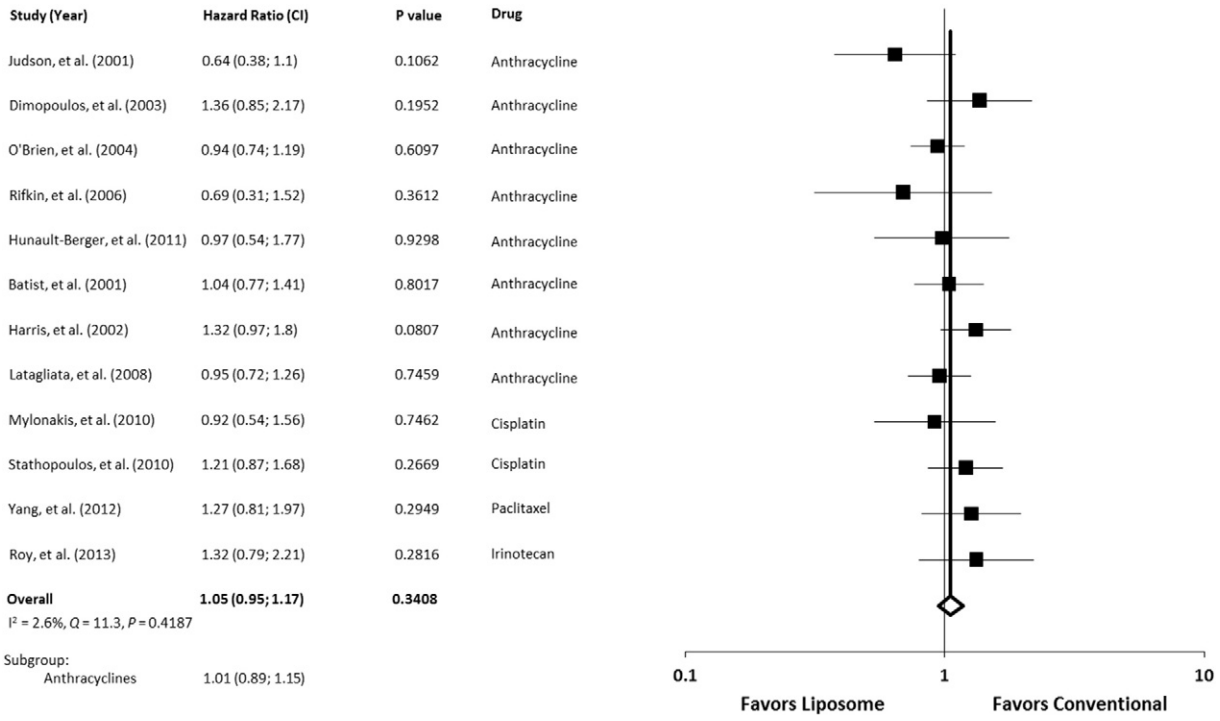

Fig. 4. Overall survival in patients treated with liposomal versus conventional chemotherapy formulations.

nanoparticles are known to interact with plasma proteins, including the complement proteins, and these interactions can alter pharmacokinetic parameters such as clearance of the delivery system [53]. Moreover, in mice treated with polymer nanoparticles, enhanced tumor growth has been linked to increased production of the anaphylatoxin C5a from C5 cleavage [51]. This phenomenon would not be discernable using DBA/2 and other C5 deficient "wild-type" mouse strains. Interestingly, only one study in our meta-analysis reported results from a placebo liposome control cohort and, although liposomes and other nanoparticles are known to have numerous immunological effects [54,55], none of the preclinical studies reported performing any immune assessments.

In our initial preclinical data search, most of the studies were excluded due to the absence of a published survival curve. The principal efficacy endpoint for these animal studies was tumor size which is in contrast to survival endpoints which are the gold-standard measurements of

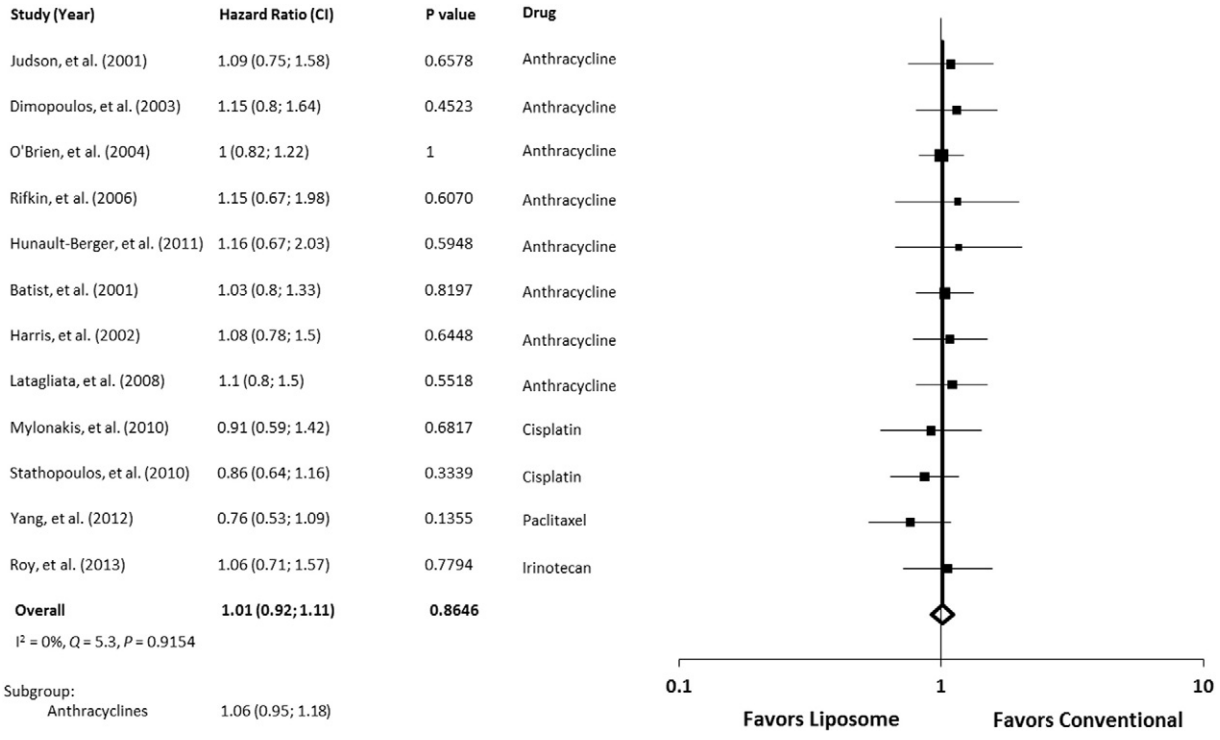

Fig. 5. Progression free survival in patients treated with liposomal versus conventional chemotherapy formulations.

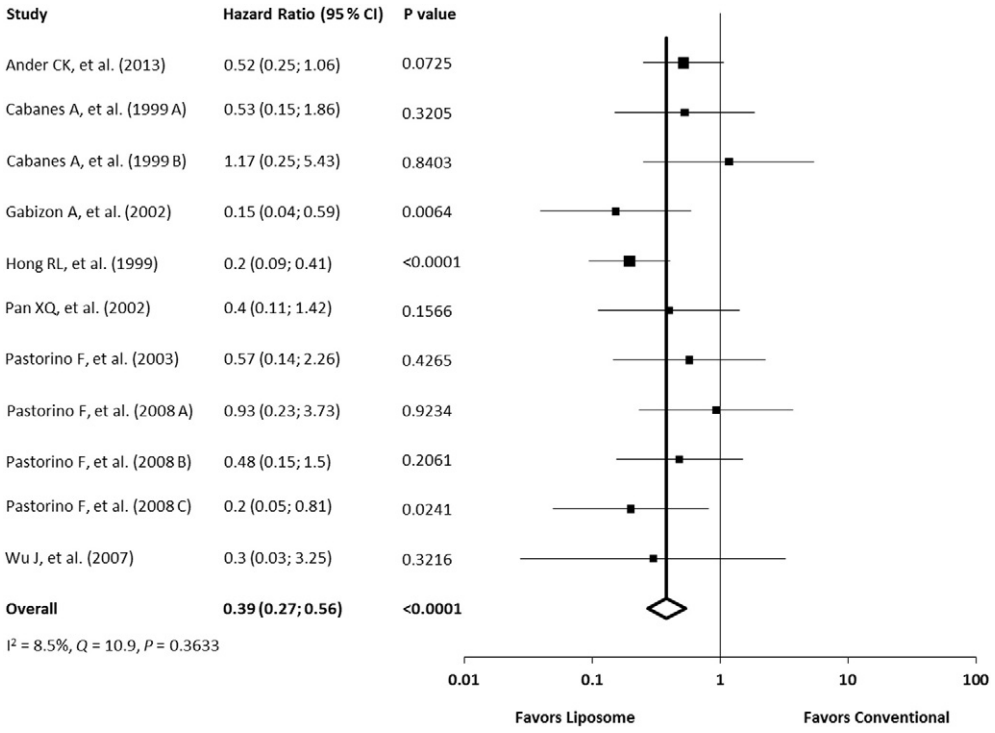

Fig. 6. Overall survival in tumor bearing mice treated with pegylated liposomal doxorubicin versus conventional doxorubicin.

efficacy in clinical trials. This could be another reason for the lack of translation of efficacy from preclinical mouse models to clinical trials. It has been shown in clinical trials that tumor response rates (e.g., size shrinkage) are not accurate predictors of survival benefit [56,57]. Yet, the predominant strategy for the preclinical development of anticancer therapies continues to rely heavily on tumor response as a measurement of efficacy. It is possible that utilization of survival as an efficacy endpoint in animal studies may be more predictive of clinical efficacy, however formal assessments of this relationship are currently lacking.

To our knowledge, we are the first to systematically compare the preclinical and clinical survival benefits of antineoplastic liposomal drugs. These initial findings should be interpreted with caution due to the high degree of heterogeneity between the preclinical studies in tumor models, treatment protocols, and endpoint assessments. This lack of standardized procedures is a major barrier to determining the translatability of preclinical efficacy endpoints and tumor models. To facilitate the translation of preclinical findings to the clinical trial setting, we propose that consensus guidelines be created for conducting and reporting of results from preclinical studies evaluating nanoparticle drug efficacy. The current preclinical development framework for antineoplastic agents includes xenograft tumor models to assess anticancer efficacy against human cancers. In addition, liposomal formulations can also significantly alter the side effect profile; liposomal doxorubicin has significantly less cardiac toxicity than conventional doxorubicin, however there is an increased incidence of acute infusion reactions and mucocutaneous toxicity. The preclinical development strategies have adapted to these new findings by including rodent models to assess the risk of cardiotoxicity [58], swine models to assess the risk of acute infusion reactions [59], and canine models have been found to reproduce mucocutaneous toxicities similar to those seen in patients [60]. Given the recent evidence showing that carrier-immune interactions can impact tumor growth, we would further recommend the inclusion of immunocompetent tumor models, inclusion of control cohorts

Table 4
Quality assessment of the included clinical trials.

Study	Sequence generation	Allocation concealment	Blinding	Incomplete outcome data	Selective outcome reporting	Intent-to-treat analysis	Other potential threats
Judson [21]	Random; unclear	Unclear	No	No	Yes	Yes	Yes
Dimopoulos [22]	Random; permuted blocks	Unclear	No	No	Yes	Yes	Yes
O'Brien [6]	Random; computer generator	Yes	No	No	Yes	No	Yes
Rifkin [23]	Random; unclear	Unclear	Evaluators blinded	No	Yes	Yes	Yes
Hunault-Berger [24]	Random; unclear	Unclear	No	No	Yes	Yes	Yes
Batist [4]	Random; balanced blocks	Unclear	Evaluators blinded	No	Yes	Yes	Yes
Harris [25]	Random; balanced blocks	Unclear	Evaluators blinded	No	Yes	Yes	Yes
Latagliata [26]	Random; unclear	Unclear	No	No	Yes	Yes	Yes
Jehn [27]	Random; unclear	Unclear	No	Yes	Yes	No	Yes
Kosmas [28]	Random; unclear	Unclear	Unclear	Yes	Yes	No	Yes
Mylonakis [29]	Random; unclear	Unclear	No	No	Yes	Yes	Yes
Stathopoulos [30]	Random; permuted blocks	Unclear	Evaluators blinded	No	Yes	Yes	Yes
Yang [31]	Random; unclear	Unclear	No	No	Yes	Yes	Yes
Roy [32]	Random; unclear	Unclear	Evaluators blinded	No	Yes	Yes	Yes

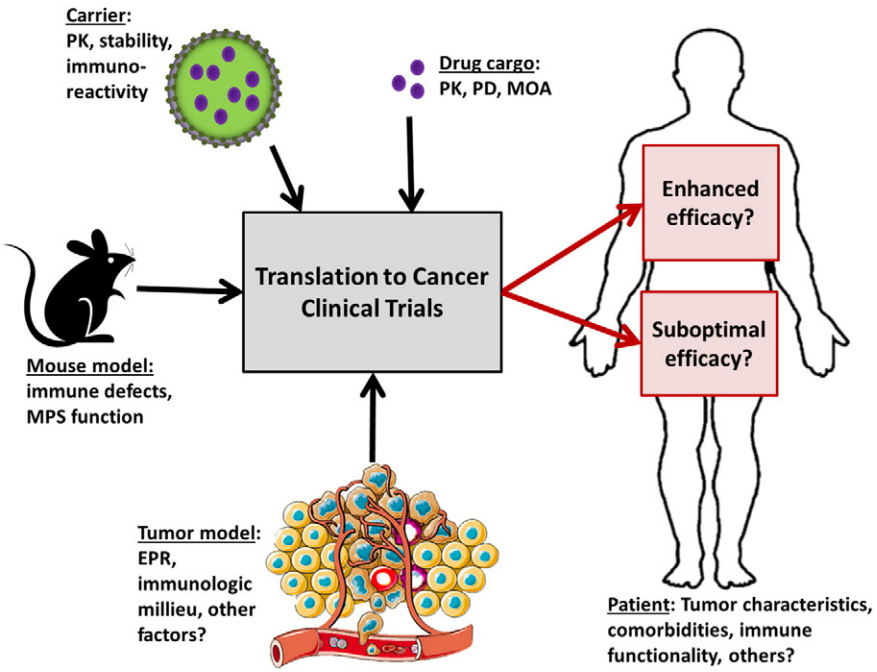

Fig. 7. Challenges associated with translating the anticancer efficacy of carrier-mediated drugs from preclinical studies to clinical trials. PK, pharmacokinetics; PD, pharmacodynamics, MOA, mechanisms of action; MPS, mononuclear phagocyte system; EPR, enhanced permeability and retention.

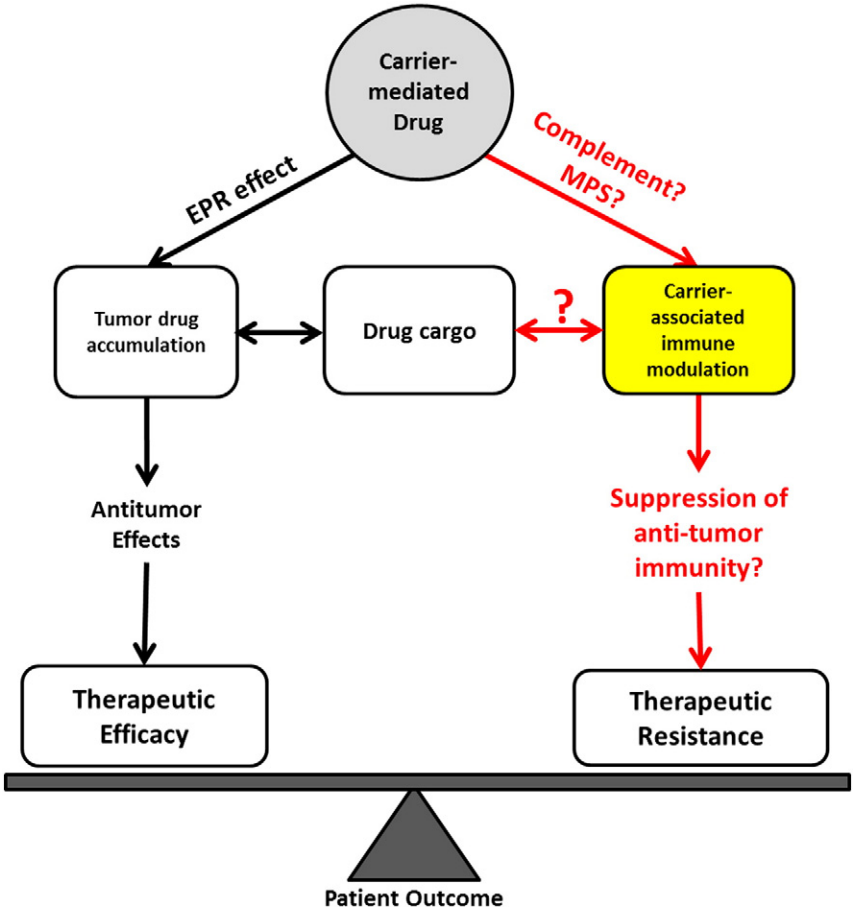

Fig. 8. A model of how interactions between liposomal drugs and the immunologic milieu may impact clinical anticancer efficacy. EPR, enhanced permeability and retention; MPS, mononuclear phagocyte system.

treated with placebo carriers, and routine in vivo assessments of immune activation and inhibition. It may even be considered prudent to include these or similar studies as prerequisites for carrier-mediated drugs seeking to enter cancer clinical trials. In addition, survival should be considered as an endpoint in preclinical studies and testing on indolent and metastatic tumor models should be encouraged.

There are several other limitations to our study. While we found that liposomal formulations were not more clinically efficacious than their conventional formulations in patients, the majority of the included trials evaluated anthracyclines (doxorubicin and daunorubicin). Further subgroup analysis of only the cisplatin trials showed that the liposomal formulation had higher response rates than conventional cisplatin. Whether this enhanced efficacy is true with regards to OS and PFS remains to be determined as we could not evaluate this due to the lack of published data on these endpoints. Moreover, all the patients in the cisplatin trials had non-small cell lung cancer thus it is possible that the improved response to liposomal drugs may be due to factors such as vascularity and immunogenicity that is associated with cancer type. Interestingly, subgroup analysis of only metastatic breast cancer patients showed no improvement in any efficacy endpoints. However, the breast cancer subgroup also comprised only liposomal anthracycline formulations. It is not possible in our meta-analysis to determine whether this difference in efficacy advantage between lung cancer and breast cancer patients is due to differences in cancer biology or due to the differences in pharmacology upon liposome-encapsulation of platinum versus anthracycline agents. Thus, additional prospective clinical trials and preclinical mechanistic studies are needed to elucidate the role of the drug cargo and the tumor microenvironment in the anticancer efficacy of liposomal drugs.

Our study focused on liposomal formulations because they are the most extensively utilized carrier platform in cancer patients and subgroup analyses of simple liposomal versus pegylated liposomal anthracyclines did not show an improvement in efficacy over conventional formulations with either type of liposomes. Since carrier physicochemical characteristics can impact their interactions with cellular and molecular components in vivo resulting in different drug release rates, tissue distribution, etc. [55,61,62], the results of our meta-analysis should not be generalized to other types of carriers. Moreover, given the complex and distinct mechanisms of action of different cytotoxic agents, our findings should not be generalized to other chemotherapeutic agents beyond anthracyclines and cisplatin. Clearly, more clinical trials directly comparing carrier-mediated and conventional chemotherapy formulations are needed. However, it is not evident that these trials will be forthcoming. Most of the trials in our initial search results were excluded because they compared liposomal formulation to standard of care regimens that did not contain the equivalent conventional formulation, and this appears to be the predominant strategy in the clinical development of anticancer nanoparticles.

5. Conclusions

The full clinical potential of carrier-mediated drugs remains to be realized and many unanswered questions persist regarding the pharmacology of these agents. The optimal dosing regimen for carrier-mediated agents has not been thoroughly investigated and it is possible that this will differ from that of the conventional formulation. Moreover, the contribution of the EPR effect and the tumor microenvironment to clinical efficacy needs to be fully elucidated. In particular the mechanisms and consequences of their interaction with the immune system in cancer are currently unclear. Finally, the development of consensus guidelines on the conduct and reporting of preclinical studies evaluating anticancer nanoparticles is needed in order to facilitate the interpretation and translation of results from animal models.

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