Drug delivery systems for ovarian cancer treatment: a systematic review and meta-analysis of animal studies

René Raavé, Rob B de Vries, Leon F Massuger, Toin H van Kuppevelt, Willeke F Daamen

Current ovarian cancer treatment involves chemotherapy that has serious limitations, such as rapid clearance, unfavorable biodistribution and severe side effects. To overcome these limitations, drug delivery systems (DDS) have been developed to encapsulate chemotherapeutics for delivery to tumor cells. However, no systematic assessment of the efficacy of chemotherapy by DDS compared to free chemotherapy (not in a DDS) has been performed for animal studies. Here, we assess the efficacy of chemotherapy in DDS on survival and tumor growth inhibition in animal studies. We searched PubMed and EMBASE (via OvidSP) to systematically identify studies evaluating chemotherapeutics encapsulated in DDS for ovarian cancer treatment in animal studies. Studies were assessed for quality and risk of bias. Study characteristics were collected and outcome data (survival/hazard ratio or tumor growth inhibition) were extracted and used for meta-analyses. Meta-analysis was performed to identify and explore which characteristics of DDS influenced treatment efficacy. A total of 44 studies were included after thorough literature screening (2735 studies found after initial search). The risk of bias was difficult to assess, mainly because of incomplete reporting. A total of 17 studies (377 animals) and 16 studies (259 animals) could be included in the meta-analysis for survival and tumor growth inhibition, respectively. In the majority of the included studies chemotherapeutics entrapped in a DDS significantly improved efficacy over free chemotherapeutics regarding both survival and tumor growth inhibition. Subgroup analyses, however, revealed that cisplatin entrapped in a DDS did not result in additional tumor growth inhibition compared to free cisplatin, although it did result in improved survival. Micelles did not show a significant tumor growth inhibition compared to free chemotherapeutics, which indicates that micelles may not be a suitable DDS for ovarian cancer treatment. Other subgroup analyses, such as targeted versus non-targeted DDS or IV versus IP administration route, did not identify specific characteristics of DDS that affected treatment efficacy. This systematic review shows the potential, but also the limitations of chemotherapy by drug delivery systems for ovarian cancer treatment. For future animal research, we emphasize that data need to be reported with ample attention to detailed reporting.

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1 Abstract

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We searched PubMed and EMBASE (via OvidSP) to systematically identify studies evaluating chemotherapeutics encapsulated in DDS for ovarian cancer treatment in animal studies. Studies were assessed for quality and risk of bias. Study characteristics were collected and outcome data (survival/hazard ratio or tumor growth inhibition) were extracted and used for meta-analyses. Meta-analysis was performed to identify and explore which characteristics of DDS influenced treatment efficacy.

A total of 44 studies were included after thorough literature screening (2735 studies found after initial search). The risk of bias was difficult to assess, mainly because of incomplete reporting. A total of 17 studies (377 animals) and 16 studies (259 animals) could be included in the meta-analysis for survival and tumor growth inhibition, respectively. In the majority of the included studies chemotherapeutics entrapped in a DDS significantly improved efficacy over free chemotherapeutics regarding both survival and tumor growth inhibition. Subgroup analyses, however, revealed that cisplatin entrapped in a DDS did not result in additional tumor growth inhibition compared to free cisplatin, although it did result in improved survival. Micelles did not show a significant tumor growth inhibition compared to free chemotherapeutics, which indicates that micelles may not be a suitable DDS for ovarian cancer treatment. Other subgroup analyses, such as targeted versus non-targeted DDS or IV versus IP administration route, did not identify specific characteristics of DDS that affected treatment efficacy.

This systematic review shows the potential, but also the limitations of chemotherapy by drug delivery systems for ovarian cancer treatment. For future animal research, we emphasize that data need to be reported with ample attention to detailed reporting.

Keywords: animal studies, drug delivery systems, meta-analysis, ovarian cancer, systematic review

2 Introduction

Ovarian cancer is the most lethal of all gynecological cancers. It is estimated that approximately 65,500 women were diagnosed with ovarian cancer and that about 42,700 women deceased due to ovarian cancer in Europe in 2012 (Ferlay et al. 2013). Conventional therapy includes neoadjuvant chemotherapy with subsequent surgical interval debulking and subsequent chemotherapy or primary surgical debulking with adjuvant chemotherapy (Vergote et al. 2011; Vergote et al. 2010). Although systemic intravenous administration of chemotherapeutics results in elimination of cancer cells, it is associated with serious shortcomings. Chemotherapeutics have a short half-life, are toxic to healthy cells and show an unfavorable biodistribution resulting in undesired side effects such as bone-marrow suppression, neuropathy, cardiotoxiticy, hair loss and nausea (Bergkvist & Wengstrom 2006; Chon et al. 2012; Love et al. 1989; Massey et al. 2014; Monsuez et al. 2010; Truong et al. 2014). Moreover, next to systemic intravenous (IV) administered chemotherapy, local intraperitoneal (IP) in combination with IV administration is applied as well and was found to increase survival time in ovarian cancer patients (Armstrong et al. 2006; Barlin et al. 2012; Jaaback et al. 2011), but these patients had more side effects. Drug delivery systems (DDS) may overcome the current disadvantages of chemotherapeutics. By encapsulating toxic chemotherapeutics, DDS are designed to increase concentrations of chemotherapeutics at the tumor site, which could eventually result in higher treatment efficacy, while simultaneously reducing exposure of chemotherapeutics to healthy cells, resulting in a therapy with reduced side effects.

To date, abundant research has been performed on DDS, which has resulted in many kinds of DDS, such as liposomes, micelles or ‘nanoparticles’ (Lammers et al. 2012; Tomasina et al. 2013), with different characteristics for treatment of various types of cancer, including ovarian cancer. Several factors may affect the efficacy of DDS. For instance, size can be of importance as for long blood-circulation times and optimal tumor penetration an optimal size range of DDS is estimated to be in the sub-100 nm, but not smaller than 6 nm to prevent unwanted removal (Perrault et al. 2009). Another parameter that is often

93 varied among DDS is PEGylation, which is intended to prevent unwanted uptake by the liver and spleen
94 by coating the surface of DDS with poly(ethylene)glycol (PEG) resulting in increased blood-circulation
95 times (Ernsting et al. 2013; Perrault et al. 2009). With increasing circulation time, increased accumulation
96 of DDS can be found at the tumor site. By the enhanced permeability and retention (EPR) effect of the
97 tumor cell aggregates, due to leaky blood vessels, DDS accumulate in the tumor area and release their
98 content, so-called passive targeting (Iyer et al. 2006). On the other hand, a more active way of targeting
99 can be achieved by conjugating anti-tumor antibodies or specific receptor ligands to the wall of capsules
100 to target tumor cells specifically (Danhier et al. 2010). The passive and active targeting strategies mainly
101 apply to intravenously (IV) administered DDS. However, as IP administered chemotherapy in
102 combination with IV administered chemotherapy is being clinically applied, DDS are also being
103 administered IP instead of IV in ovarian cancer (Gunji et al. 2013; Ye et al. 2013; Zhang et al. 2013),
104 introducing another variable in DDS that can affect the efficacy of DDS therapies. Furthermore, the DDS
105 preparation material can be varied from metals to polymers to proteins, which influences properties such
106 as biodegradability, immunogenicity and toxicity, but also drug release characteristics or cellular uptake
107 of DDS. Various chemotherapeutics are entrapped in DDS for ovarian cancer treatment, such as cisplatin,
108 paclitaxel or doxorubicin, affecting the outcome of DDS treatment as well. All in all, preclinical studies
109 showed that many parameters can be varied in DDS. It is still unclear, however, which variant is most
110 effective.
111
112 The majority of DDS are evaluated *in vitro* before being tested in animal models using different cancer
113 cell lines. *In vivo* evaluation has shown a wide range of therapeutic efficacies, with different treatment
114 regimes and several time periods. Several reviews describe possible improved efficacies that
115 chemotherapy by DDS may have in animal models for cancers such as breast cancer (Yezhelyev et al.
116 2006), lung cancer (Loira-Pastoriza et al. 2014), melanoma (Cheng et al. 2014), brain cancer (Chen et al.
117 2013), colorectal cancer (De Smet et al. 2013) and ovarian cancer (Tomasina et al. 2013). A recent
118 literature overview by Tomasina *et al.* showed a number of DDS that have been studied for ovarian

cancer treatment (Tomasina et al. 2013). However, no systematic assessment of the efficacy of DDS in experimental ovarian cancer, or other cancer types, and the effects of the different characteristics of these DDS on treatment outcome has been reported. Therefore, we have conducted a systematic review of animal studies in order to gain insight into the effectiveness of the many types of DDS tested for ovarian cancer treatment.

In clinical studies, systematic reviews are common practice and they are also gaining popularity in preclinical (animal) studies. Compared to narrative reviews, systematic reviews are more structured and more thorough, resulting in a more comprehensive and transparent overview. Systematic reviews are therefore an ideal method for gaining a better understanding of the role DDS play in ovarian cancer therapy. Furthermore, such review may give new insights into the most effective capsule characteristics, how to improve the use and design of animal models, and eventually clinical trials. Moreover, meta-analysis can be used as an additional tool in systematic reviews of animal studies. While in meta-analyses of clinical data the primary goal is mostly to obtain a precise estimate of the overall effect of a certain intervention, in meta-analyses of animal studies the exact overall effect size may not be that informative (because of the often large heterogeneity between animal studies) and therefore the goal is of explorative nature to identify factors that affect the main outcome (Hooijmans et al. 2014a).

In this article, we report the results of the first systematic review of DDS evaluated in ovarian cancer animal models. In a comprehensive literature screening, we included all animal studies that used chemotherapeutics encapsulated in a DDS and evaluated their therapeutic efficiency in an orthotopic ovarian cancer animal model. A complete overview of the available literature including an assessment of the risk of bias of the individual studies is included. Where possible, meta-analyses were performed to study the extent to the efficacy of DDS depend on the different subgroup characteristics (type of drug delivery system, targeted vs. non-targeted DDS, IP vs. IV administration, type of xenografted cell line and type of chemotherapeutic in DDS).

145

3 Methods

3.1 Search strategy, inclusion and exclusion criteria

To include as many animal studies as possible on drug delivery systems for ovarian cancer treatment, a comprehensive search strategy for PubMed and EMBASE (via OvidSP) was developed. The search strategy consisted of three specific search components addressing: 1) drug delivery systems; 2) ovarian cancer; and 3) animal studies. The search strategy included thesaurus terms and keywords on the subject of drug delivery systems and ovarian cancer (see supplemental methods for complete search strings). To include all animal studies, previously developed PubMed and EMBASE search filters were used (de Vries et al. 2014; Hooijmans et al. 2010). No language restrictions were applied.

After the search strategy had been executed in PubMed and EMBASE (search up until September 1th 2014), duplicates were manually removed and the resulting studies were screened by title and abstract according to the following exclusion criteria: 1) not ovarian cancer; 2) no drug delivery system; 3) no primary study (*i.e.* only research articles, no reviews); and 4) not an animal study. Studies were classified as either included, more information required, or excluded. Included and more information required classified studies were subjected to a full text screening using the following exclusion criteria: 1) not ovarian cancer (other type of cancer cell line/primary culture); 2) ovarian cancer in other area than peritoneal cavity or ovaries (*e.g.* subcutaneous); 3) drug is not encapsulated in a particle (*e.g.* drug-particle conjugate); 4) drug antibody conjugate; 5) no drug delivery system (*e.g.* free drugs); 6) no chemotherapeutic drug; 7) gene therapy (including siRNAs, immune therapy, hormone therapy or anti-angiogenesis therapy); 8) not a primary study (*i.e.* only research articles, no reviews); and 9) not an animal study (*e.g.* human or *in vitro* study). Screenings were performed independently by two reviewers (RR and WD) using Early Systematic Review Software 2.0 (EROS, Institute of Clinical Effectiveness and Health Policy, Buenos Aires, Argentina). Differences in classification between reviewers were discussed until consensus was reached. Studies in a language other than English (*e.g.* Japanese and Chinese) were

171 screened by title and abstract by native speakers for that specific language. If a non-English study was
172 included in the systematic review, it was professionally translated by "Radboud in'to Languages"
173 (Radboud University, the Netherlands).
174

175 3.2 Study characteristics

176 Journal and author information from all included studies was registered. Drug delivery characteristics
177 (*e.g.* material, size, etc.), animal model information (*e.g.* species, cell lines, etc.) and treatment and
178 outcome characteristics (*e.g.* dose, regime, tumor size evaluation, etc.) described in Table 1 were
179 extracted. Conference abstracts and studies without data comparing free drug vs. encapsulated drug were
180 not included in the meta-analyses. One study (Ueno 1988) was not included in the meta-analysis as we
181 were not able to identify the specific inoculation area (subcutaneous or intraperitoneal).
182

183 3.3 Risk of bias analysis

184 To gain insight into the methodological quality of the included studies, we performed a risk of bias
185 assessment according to an adapted version of the risk of bias tool developed by Hooijmans *et al.* 2014
186 (Hooijmans et al. 2014b). Questions regarding reporting of randomization, blinding and sample size
187 calculation were added to the items from the risk of bias tool (see supplemental methods for complete
188 list). The complete list included 12 questions about the study quality such as "Was the allocation
189 adequately concealed?' and "Were incomplete outcome data adequately addressed?". Since we were only
190 interested in the *in vivo* experiments, we focused on these experiments for this assessment. Risk of bias
191 assessment was performed by two reviewers independently (RR and WD). Differences in assessment
192 between the reviewers were discussed until consensus was reached.
193

3.4 Data extraction and statistical analyses

For statistical analysis, two outcome measures that were presented frequently among the included studies were selected; survival (time-to-event data) and tumor growth inhibition.

Studies presenting survival data included experiments that show differences in survival of animals during the course of the study between the treatment conditions; chemotherapeutics administered in a DDS and chemotherapeutics administered without a DDS (free drug control). Tumor inhibition data was expressed in the studies as decrease in tumor size measured by, for instance, tumor weight or bioluminescence signal from the inoculated ovarian cancer cells.

To compare each study's result, data was extracted from the included studies. From experiments with survival data, individual time-to-event data was extracted and from experiments with tumor growth inhibition data we extracted the raw data such as tumor weight or bioluminescence signal. If these data were only depicted graphically, authors were contacted by e-mail to provide the numerical data. If the requested data could not be provided, we extracted individual time-to-event survival data or tumor growth inhibition means with SD and the number of animals using ImageJ (1.46r, National Institutes of Health, USA).

Since raw time-to-event survival data by themselves cannot be used for meta-analysis, hazard ratios were calculated. Hazard ratios represent the risk of dying over the course of the experiment. A hazard ratio >1 indicates that animals have a higher chance of dying due to their experimental condition, while a hazard ratio <1 indicates that animals have less chance of dying over the course of the experiment due to their treatment condition. If numerical hazard ratios were presented in included studies, they were used directly without further processing for meta-analysis. All graphically extracted survival data were first analyzed using SPSS Statistics 20.0.01 software (IBM, Amsterdam, the Netherlands). Log-hazard ratios and

219 standard errors were determined using a Cox regression analysis with treatment conditions set as
220 categorical covariates. Free drug control conditions (chemotherapeutic not in a DDS) were set as
221 reference category. To compare results between studies with tumor growth outcome measures, data were
222 translated into standardized mean differences (SMD; experimental group mean minus control group mean
223 divided by the pooled standard deviations of the two groups). A negative SMD indicates a larger
224 inhibition of tumor growth due to treatment with DDS compared to free drugs (not in a DDS), while a
225 positive SMD value indicates that treatment with free drugs is more effective. Means, standard deviations
226 (SDs) and the number of animals were extracted from the experiments and used to calculate SMDs.
227
228 Meta-analyses were performed using Review Manager Version 5.1 (Copenhagen, The Nordic Cochrane
229 Centre, The Cochrane Collaboration, 2011). Two separate meta-analyses were performed for the outcome
230 measures survival and tumor growth inhibition. For time-to-event data (survival), a (generic) inverse
231 variance model with random effects and hazard ratio as effect measure was applied. In this model, the
232 extracted log-hazard ratios with standard errors from the studies were entered in Review Manager and
233 used to calculate hazard ratios with 95% confidence intervals for the meta-analysis. For tumor growth
234 inhibition data, a (continuous) inverse variance model with random effects and standardized mean
235 difference as effect measure was used. If the same study included more than two experimental conditions,
236 the separate experiments were included in the meta-analysis. If in these cases there was only one control
237 condition, the *n* for the control condition was adjusted by dividing it by the number of included
238 conditions, to prevent that animals were included more than once in the meta-analysis. I^2 was used as a
239 measure of heterogeneity. In order to explore potential causes of heterogeneity, subgroup analyses were
240 planned for 1) drug delivery system, 2) chemotherapeutic used, 3) xenografted cell line in animal model,
241 4) targeted vs. non-targeted and 5) IP vs. IV administered DDS. Because of a lack of power, subgroups
242 containing less than three experiments were not used for subgroup analysis. To further investigate the
243 effect of individual experiments on the overall effect or on subgroup effects, sensitivity analyses were

244 performed by checking whether the direction of the overall or subgroup effect and their confidence
245 intervals altered substantially when individual experiments were removed from the meta-analyses.
246
247 Furthermore, to identify possible publication bias (an underrepresentation of small studies with neutral or
248 negative effects), a funnel scatter plot with the studies' intervention effect on the horizontal axis and the
249 studies' standard error on the vertical axis was created and evaluated.
250

4 Results

4.1 Study inclusion and characteristics

Search strategies designed to include animal studies about ovarian cancer and treatment using drug delivery systems resulted in a total of 2735 studies, whereof 1682 and 1053 from EMBASE and PubMed, respectively (Fig. 1). After removal of duplicates, 1947 studies were screened by title and abstract, which resulted in removal of 1682 studies. Subsequently, 265 studies were screened by full text. Of the studies screened by full text, 221 studies were excluded and 44 were included in this systematic review. The major reason for excluding studies was the use of a clinically irrelevant animal model ("ovarian cancer cells used in other area than peritoneal cavity or ovaries").

The characteristics of the included studies are summarized in supplementary table 1. Many different DDS were designed and used to treat ovarian cancer *in vivo*. Most studies (36%) used liposomes to encapsulate a chemotherapeutic drug. Approximately 16% of the studies used micelles while others used capsules labeled as nano- or microparticles (9% and 27%, respectively). Furthermore, studies that used nanogels, nanosuspensions, microbullets, virus cages and nanobins were included as well. Preparation material varied among the different designed DDS as shown in supplementary table 1. Active targeting to ovarian cancer cells using antibodies and receptor ligands such as HER-2 (Cirstoiu-Hapca et al. 2010), OV-TL3 (Storm et al. 1994; Vingerhoeds et al. 1996), folate (Chaudhury et al. 2012; Tong et al. 2014; Werner et al. 2011; Zeng et al. 2013) or luteinizing hormone-releasing hormone analogs (Pu et al. 2014) conjugated to the DDS were used in 13 out of the 44 studies (30%).

Several studies applied specific modifications to create a triggered drug-release. Gilmore *et al.* prepared nanoparticles from an acrylate monomer using a mini-emulsion polymerization technique to create particles that are stable at neutral pH and expand after endocytosis at low pH to release their payload (Gilmore et al. 2013; Griset et al. 2009). Xu *et al.* prepared cisplatin nanoparticles from poly[2-(N,N-

diethylamino)ethyl methacrylate]-block-poly(ethylene glycol) using a solvent-displacement method that also released its payload at low pH (Xu et al. 2006). Moreover, using a poly-isobutylene-maleic-glucosamine cisplatin combination, an acid-triggered drug delivery system was developed and probed to treat ovarian cancer by Paraskar *et al.* and Sengupta *et al.* (Paraskar et al. 2010; Sengupta et al. 2012).

Other modifications were applied to ensure specific delivery and release of anti-tumor drug to ovarian cancer cells and thus to increase the efficiency of the DDS *in vivo*. Lu *et al.* designed two types of tumor penetrating microparticles from poly(DL-lactide-coglycolide) that could either prime tumors with a rapid release, or sustain a specific drug level using a slow release microparticle (Lu et al. 2008). Others applied a post-ultrasound strategy to release the chemotherapeutic drug from micelles or to facilitate intracellular drug uptake from microbubbles upon injection (Gao et al. 2005; Pu et al. 2014; Rapoport et al. 2004). In these studies, the ultrasound (30 sec, 1 to 3 MHz by Rapoport *et al.* and Gao *et al.* and 3 min, 0.3 MHz by Pu *et al.*) was applied through a coupling gel to the abdominal area.

Frequently used clinically approved chemotherapeutic agents for ovarian cancer treatment doxorubicin, cisplatin and paclitaxel were used in 12, 7 and 16 studies, respectively. The remainder used other chemotherapeutic agents as described in supplementary table 1. One study applied co-delivery of doxorubicin and irinotecan using liposomes (Javid et al. 2014).

Other smaller parameters were applied to the DDS as well. About one third (14 studies) of the included studies applied PEGylation to prolong circulation time. The route of application was varied among the included studies. DDS were either administered intraperitoneally (30 studies), intravenously (8 studies), or a combination of both (6 studies).

Three of the 44 studies used a rat (Fisher F344, female) model in combination with the NuTu19 rat ovarian cancer cell line, while the remaining 41 studies (93%) used a mouse model that was either female

(28 studies), male (1 study), a combination of male and female (1 study) or not described (11 studies). Within the mice studies, the strains and genotypes varied a lot of which an athymic or nude (*Foxn1*^{nu}) mice lacking T-cells was most frequently used (28 studies). Another mouse model lacking both B- and T-cells, the severe combined immunodeficient (*scid*) model, was used in 6 of the included studies. The remaining studies used non-specified C57BL6 mice (Alagkiozidis et al. 2009; Yang et al. 2014), a non-defined Balb/c immunodeficient model (Javid et al. 2014), FVB mice (Mantia-Smaldone et al. 2014) or did not describe details about the strain (Winer et al. 2010). Moreover, two included studies from Pataskar *et al.* (Paraskar et al. 2010) and Sengupta *et al.* (Sengupta et al. 2012) used a non-xenografted adenovirus Cre recombinase induced K-ras$^{LSL/+}$Pten$^{FL/FL}$ mutated ovarian cancer mouse model. Among the xenografted mice models, most were inoculated with well-established ovarian cancer cell lines OVCAR-3 (11 studies) or SKOV-3 (10 studies). Different cell numbers were inoculated in the mice, but a number of $5 \cdot 10^6$ cells was most frequently used. Most studies used animals that were approximately 4-8 weeks old (23 studies), although 18 studies did not describe the age of their animal model.

4.2 Risk of bias assessment

Figure 2 provides an overview of the risk of bias assessment of the 44 included studies (for scores per individual study see supplemental material). From questions 1-3 (Fig. 2), it can be seen that only 39% and 9% of the studies mentioned any kind of randomization or blinding, respectively. In none of the studies, a power calculation was shown or mentioned.

Questions regarding allocation and correction for baseline differences (Fig. 2 question 4-6) showed that one study did not adequately apply the allocation sequence, while for the others it was unclear. In three studies (7%), groups were not similar at the baseline or they did not correct for confounders, while 5 studies (11%) did correct for baseline differences or confounders. None of the studies described if and how allocation was concealed during the experiment (question 6). In one study, it was clear that the

327 animals were not randomly housed during the experiment (question 7), for the others it was unclear. A
328 similar result was found regarding blinding during the experiment (question 8); only one study described
329 details about blinding during the experiment, which suggested, however, that this was not performed
330 adequately. Regarding outcome assessment, two studies (5%) selected animals at random for outcome
331 assessment and one study (2%) blinded the outcome assessor. Two studies (5%) did not correctly handle
332 incomplete outcome data because differences between the number of animals at the start of the
333 experiment and the number of animals in the analysis were left unexplained. Finally, 9% of the included
334 studies were not free of other problems that could induce a bias. Most of these studies described a
335 potential conflict of interest. A general observation in our risk of bias assessment was that the majority of
336 the included studies did not provide sufficient information to assess the risk of bias. The studies did not
337 adequately describe details regarding allocation of animals to the experimental groups, adjustments for
338 baseline differences, concealment of allocation, randomization, blinding and addressing incomplete
339 outcome data.
340

341 ### 4.3 Meta-analyses

342 Two types of outcome measures were frequently described in the included studies: survival and tumor
343 growth inhibition. In order to obtain a general idea of the direction of the outcome of the different studies,
344 meta-analyses were performed for these outcome measures separately.
345

346 #### 4.3.1 Survival

347 ##### *4.3.1.1 Forest plots*

348 18 studies described results with survival data. These data were used to calculate hazard ratios. A total of
349 30 experiments were suitable for performing a meta-analysis, which represented 377 animals. From these
350 30 experiments, 12 experiments showed a significantly decrease in hazard ratio, while one experiment

showed a significant increase in hazard ratio (Fig. 3a). This may indicate that treatment of animal models for ovarian cancer with chemotherapeutics in a DDS is more effective in preventing death than treatment with free chemotherapeutics. For four studies (due to small group numbers) no models could be fitted, which resulted in a hazard ratio of 0 with a very wide confidence interval.

4.3.1.2 Type of DDS

As shown in Figure 3b, a subgroup analysis was performed to evaluate the overall effect of experiments that used liposomes (12 experiments) or micro/nanocapsules (15 experiments). No difference in effect on hazard ratio was found between experiments that used liposomes or micro/nanocapsules; all resulted in a significant decrease of the hazard ratio.

4.3.1.3 Type of chemotherapeutic

To investigate whether different tumor drugs encapsulated in DDS affect the hazard ratio, subgroup analysis by chemotherapeutic cisplatin (7 experiments), doxorubicin (4 experiments) and paclitaxel (16 experiments) was performed (Fig. 3b). Cisplatin, doxorubicin and paclitaxel all resulted in a significant decrease in hazard ratio. No significant differences were observed among the three drug subgroups.

4.3.1.4 Targeting vs. non-targeting

Drug delivery systems targeted specifically (12 experiments) to ovarian cancer cells did not result in a lower hazard ratio compared to non-targeted DDS (18 experiments). Both treatment strategies resulted in a lower subtotal hazard ratio, suggesting that both targeted and non-targeted DDS treatment result in improved survival rates (Fig. 3b).

4.3.1.5 Route of administration

A subgroup analysis of the different routes of administration was performed to explore whether this would affect the treatment outcome. Both IP (17 experiments) and IV (7 experiments) administration significantly lowered the risk of dying over time (Fig. 3b). Moreover, experiments that used a

375 combination strategy of IP and IV treatment (6 experiments) also resulted in a lower hazard ratio. No
376 statistical differences between IV, IP or a combination of IV and IP administration were observed.
377

378 *4.3.1.6 Applied xenografted cell line*
379 Ovarian cancer cell lines SKOV-3 (9 experiments), OVCAR-3 (5 experiments), A2780 (7 experiments),
380 ID-8 (3 experiments) and IGROV-1 (3 experiments) subgroups could be included in the subgroup
381 analysis as these had ≥3 studies in the several subgroups. This meta-analysis showed that mice
382 xenografted with SKOV-3, OVCAR-3 and ID-8 followed by treatment with chemotherapeutics had a
383 significant decrease in hazard ratio (Fig. 3b). Mice xenografted with IGROV-1 or A2780 that were treated
384 with DDS did not significantly benefit from DDS treatment compared to free drug controls.
385

386 4.3.2 Tumor growth inhibition

387 *4.3.2.1 Forest plot*
388 A total of 16 studies presented data regarding tumor growth inhibition using a drug delivery system
389 compared to a free drug control. From these studies, 21 experiments could be used for meta-analysis
390 representing a total of 259 animals. Nine of the experiments showed a statistically significant result to the
391 effect that chemotherapeutics in DDS inhibit tumor growth better than free drugs (Fig. 4a). The study of
392 Konishi *et al.* reported a significant tumor growth inhibition. However, this could not be included in the
393 meta-analysis due to the absence of a standard deviation in the experimental group. No studies reported
394 significantly more tumor growth inhibition by free drug treatment compared to the DDS treatment. These
395 results suggest that chemotherapeutics in a DDS in general have a higher efficacy regarding tumor growth
396 inhibition than free chemotherapeutics.
397

398 *4.3.2.2 Type of DDS*

399 To gain insight in the effectiveness of different types of DDS, a subgroup analysis by DDS type was
400 performed (Fig 4b). A statistically significant difference between the subgroups micro/nano-particles (13
401 experiments) and micelles (3 experiments) was observed; treatment with micro/nano-particles seemed to
402 perform better than treatment with micelles. On the other hand, no significant difference between the
403 results of liposomes (3 experiments) and micro/nanoparticles was found.

404

405 *4.3.2.3 Type of chemotherapeutic*

406 Subgroup analysis of tumor growth inhibition data by anti-tumor drug was possible for the
407 chemotherapeutics cisplatin (7 experiments) and paclitaxel (9 experiments) with 7 and 9 experiments,
408 respectively (Fig. 4b). Surprisingly, cisplatin encapsulated in DDS did not result in enhanced tumor
409 growth inhibition compared to free drug control, whereas encapsulated paclitaxel was much more
410 effective than free paclitaxel. Moreover, the difference between subgroups paclitaxel and cisplatin was
411 statistically significant.

412 *4.3.2.4 Targeted vs. non-targeted*

413 Non-targeted DDS reach tumor cells passively by exploiting the leaky vessels of the tumor vasculature.
414 On the other hand, DDS can be decorated with tumor-specific antibodies or receptor-ligands to actively
415 target tumor cells. A subgroup analysis for targeted (4 experiments) vs. non-targeted (17 experiments)
416 DDS showed that both targeted and non-targeted DDS could significantly inhibit tumor growth more
417 compared to their free drug controls (Fig 4b). However, no significant difference was observed between
418 the targeted and non-targeted subgroups.

419 *4.3.2.5 Route of administration*

420 A total of 16 experiments administered their treatment IP, while 4 experiments used an IV strategy. Both
421 routes seem to be effective, but no statistical difference in effectiveness between the two routes was
422 found, suggesting that IP administration of DDS has no advantage over IV in animals.

423

424 4.4 Sensitivity analysis

425 To assess the robustness of the meta-analyses' results, a sensitivity analysis was performed. This analysis
426 assessed the influence of individual studies with their specific experimental set-up (*e.g.* number and type
427 of inoculated ovarian cancer cells, treatment dose and regime, or genotype differences) on the overall
428 outcome effect.

429

430 4.4.1 Survival data

431 It was investigated whether studies that had dose differences between the DDS and free drug groups
432 (marked with an asterisk in Figure 3 and 4) affected the overall effect. Exclusion of these studies,
433 however, did not affect the direction of the overall effect.

434

435 For experiments from Chaudhury *et al.* (one experiment), Cirstoiu-Hapca *et al.* (two experiments), and
436 Yang *et al.* (one experiment), it was not possible to accurately estimate a hazard ratio from the log-hazard
437 ratios. In these experiments, there was not enough information (*e.g.* only one event over the course of the
438 experiment) to converge and fit a model. This resulted in a hazard ratio of 0 with a very wide confidence
439 interval. Excluding these experiments from the analysis hardly had any effect on the overall outcome.

440

441 4.4.2 Tumor growth inhibition data

442 For tumor growth inhibition data, experiments from Javid *et al.* and Lu *et al*. 2007 showed extremely high
443 tumor growth inhibition for their DDS groups. Therefore, we wondered whether the overall positive effect
444 was caused by these experiments. However, these studies did only affect overall tumor growth inhibition
445 to a small extent; a meta-analysis without these studies still resulted in a significant inhibition of tumor
446 growth due to treatment with chemotherapeutics entrapped in a DDS.
447
448 Li and Howell, and Patankar *et al.* used different doses of chemotherapeutics in the treatment group and
449 control group. Therefore, it was tested whether these studies were responsible for the positive overall
450 outcome. However, excluding these studies did not affect the overall meta-analysis effect size.
451
452 Moreover, it was investigated whether two studies that used a rat model instead of a mouse model
453 influenced the overall outcome (Ye *et al.* 2013 and Lu *et al.* 2007). A meta-analysis without these rat
454 studies still resulted in an overall significant inhibition of tumor growth for animals treated with
455 chemotherapeutics in a DDS compared to animals treated with free chemotherapeutics.
456

457 4.5 Publication bias assessment

458 Publication bias was assessed for the time-to-event outcome measure, since this outcome measure
459 included the largest number of studies. To investigate publication bias, a funnel plot was created (Fig. 5).
460 The experiments with almost infinite confidence intervals (Chaudhury *et al.*, Cirstoiu-Hapca *et al.* and
461 Yang *et al.*,) were not included in the funnel plot as these would introduce a very large y-axis interval
462 making the graph unclear. The funnel plot indicated missing studies at the right bottom side of the overall
463 effect where small studies with a high hazard ratio (less survival in DDS group) would be expected,
464 suggesting publication bias.

5 Discussion

This systematic review was performed to investigate the effect of chemotherapeutic-DDS and their specific characteristics on ovarian cancer treatment in animal models. We looked at two outcome measures; survival and tumor growth inhibition, which resulted in meta-analyses of 17 and 16 studies that included 377 and 259 animals, respectively. Overall, the majority of the studies showed that treatment with chemotherapeutics entrapped in DDS used for *in vivo* treatment of experimental ovarian cancer had better efficacies on both survival and tumor growth inhibition compared to chemotherapeutics not entrapped in a DDS. This result is to some extent similar to what is found in clinical studies, which observed increased efficacy of doxorubicin in a DDS (pegylated liposomes) either in different staged ovarian cancer patient groups or compared to different treatment regimes with other chemotherapeutics. Although these studies did not compare free doxorubicin and doxorubicin by a DDS, most consider pegylated liposomal doxorubicin as a safe and effective treatment (Gordon et al. 2000; Muggia et al. 1997; Safra et al. 2001; Uziely et al. 1995).

However, a few observations in the field of drug delivery and ovarian cancer treatment were not supported by our results. Our results in animal studies do not show that one administration route (either IV, IP or a combination of both) had an advantage over another route looking at tumor growth inhibition and survival. This seems to be in contrast with clinical data where several lines of evidence suggest that treatment of ovarian cancer patients with a combination of IP and IV treatment with free chemotherapeutics may be more effective than IV treatment only (Jaaback et al. 2011). It should be taken into account that these clinical studies were not performed with DDS and always included an additional systemic chemotherapy over the IP therapy. This may explain the lack of improved efficacy by IP treatment over IV treatment in our meta-analysis.

An interesting observation is that our results suggest that cisplatin, a first choice chemotherapeutic for ovarian cancer treatment, may not be a suitable candidate for treatment of ovarian cancer using DDS,

since cisplatin in DDS did not lead to more tumor growth inhibition than free cisplatin. However, this was not the case for survival, a clinically more important outcome measure, where all chemotherapeutics in DDS resulted in a significant improvement of survival compared to free chemotherapeutics. It should be noted that results from tumor growth inhibition and survival outcome measures were mostly not based on data from the same studies. Interestingly is that in a phase II clinical study evaluating liposomal cisplatin a lack of clinical response was observed (Seetharamu et al. 2010). Moreover, in 1998, Sugiyama *et al.* evaluated microspheres containing cisplatin compared to an aqueous solution of cisplatin and found in a small ovarian cancer patient group similar toxicity profiles, but no data on efficacy was shown (Sugiyama et al. 1998). No subsequent phase I/II clinical trials of this DDS regarding ovarian cancer treatment could be identified in current literature, which may suggest a possible lack of clinical outcome. These two cisplatin DDS examples may confirm our results that cisplatin may not be the most suitable drug to be used in a DDS for ovarian cancer treatment.

Our results show that animal studies do not indicate higher treatment efficacies by active targeting, as both active and passive targeting resulted in almost similar inhibition of tumor growth and improved survival in animal studies. This seems to be in contrast with the current direction of the drug delivery research field where an important goal in the development of DDS is to improve treatment efficacy and simultaneously decrease side effects of chemotherapeutics. By active targeting of tumor cells with antibodies or tumor receptor ligands attached to DDS, it is hypothesized that these DDS only bind to tumor cells and not to healthy cells, thereby improving treatment efficacy and simultaneously decreasing side effects (Bae & Park 2011). All 7 included studies in our systematic review that evaluated chemotherapy both targeted and non-targeted DDS did not show significant differences between chemotherapy by targeted or non-targeted DDS our survival or tumor reduction meta-analyses. However, if targeted therapy would show an advantage over non-targeted therapy, such as fewer side effects, chemotherapy by targeted DDS would be preferable over chemotherapy by non-targeted DDS. From the 7 included studies that tested chemotherapy by both targeted and non-targeted DDS, only four studies

517 mentioned that side effects were evaluated. None of these studies reported differences between targeted
518 and non-targeted DDS. As our results showed no advantage of targeted DDS, although with limited
519 power, we therefore carefully hypothesize that chemotherapy by targeted DDS may have no or only little
520 advantage over chemotherapy by non-targeted DDS when only looking at tumor growth inhibition and
521 survival. Future animal studies investigating differences between chemotherapy by targeted and non-
522 targeted DDS should be performed to show the advantages of targeted DDS.
523
524 Looking at tumor growth inhibition, our analysis suggested that micro/nanoparticle DDS are most
525 efficient and significantly better than micelles. Micelles do not result in significant tumor growth
526 inhibition, which suggests that micelles may not be the most suitable DDS for chemotherapeutic ovarian
527 cancer treatment. This could not be confirmed with survival data, as the micelles subgroup contained too
528 little experiments. The two experiments evaluating micelles and showing survival data both did not show
529 a significant improved hazard ratio. Future research should therefore show whether chemotherapy using
530 micelles would improve survival outcome. Moreover, we would like to emphasize that the
531 micro/nanoparticle group was very heterogeneous. However, making subgroups of the micro/nanoparticle
532 group was not feasible due to the lack of experiments performed with each specific DDS. Therefore, more
533 experiments containing direct comparisons would be needed to demonstrate that a specific type or class of
534 DDS has the best efficacy.
535
536 We tried to investigate the role of the ovarian cancer animal model. During the screening of studies for
537 inclusion in this systematic review, we came across many animal studies that used a less physiologically
538 relevant subcutaneous animal model (Vanderhyden et al. 2003). As these animal models do not reflect the
539 disease progression of ovarian cancer, we decided to focus only on studies that used a clinically important
540 orthotopic intraperitoneal ovarian cancer animal model. This decision may explain why our results are
541 less positive than the current direction in literature (*e.g.* no advantage of targeted DDS).
542

543 It is interesting that there is no consensus about the specific cell line used for the assessment of DDS
544 efficacy. Domcke *et al.* evaluated the genetic differences between cell lines and original tumor tissue
545 (Domcke et al. 2013). Most frequently used ovarian cancer cells lines such as SKOV3, A2780 and
546 IGROV-1 may not be suitable models for ovarian carcinoma cell lines and results from experiments with
547 these cell lines should therefore be interpreted with caution, especially when translating these results to
548 the clinic.
549
550 Our results showed no significant improved survival in animal models with A2780 or IGROV-1 cell
551 lines. They may be considered to be poor models for ovarian cancer, but there are no explanations that
552 these cell lines would be less sensitive for chemotherapy by DDS. Despite to their lack of clinical
553 representativity, we have no reasons to prefer a certain cell type for experiments regarding chemotherapy
554 by DDS based on results from this systematic review and meta-analysis.
555
556 We want to mention a number of limitations of this review. Both the overall analysis and the subgroup
557 analyses displayed relatively high levels of heterogeneity, even though the levels within the subgroups
558 were somewhat lower than in the overall analysis. Because of this (expected) heterogeneity, the meta-
559 analyses were used to explore potential characteristics of DDS that affect final outcome in a hypothesis-
560 forming rather than hypothesis-confirming manner.
561
562 Another limitation is the lack of response from authors from included studies when asked to share their
563 raw data. As only a few authors were willing to share their raw data, we had to extract raw data from most
564 included studies manually. Although performed carefully, this may have introduced small errors in the
565 data used for meta-analyses.
566
567 The possibility of bias in the included studies in this systematic review may have introduced an
568 overestimation of the meta-analyses' results. The reliability of the results of a systematic review greatly

depends on the quality of the included studies. Unfortunately, most studies lacked reporting of important details in their experimental set-up. Therefore, it was difficult to assess whether studies actually had a low or high risk of bias. To compare efficacies of chemotherapeutics in DDS compared to free chemotherapeutics, the experimental set-up is of major importance. For instance, blinding and randomization contribute to the overall validity of the experimental set-up (Hirst et al. 2014). Most studies used humane endpoints for the sake of the animals' welfare. However, if not blinded, one can imagine that control animals may be considered to meet humane endpoint criteria earlier (Bello et al. 2014), which may introduce a bias in the outcomes of the study, particularly if survival is an outcome measure. Moreover, almost all studies used a xenografted animal model that was first inoculated with cells before treatment initiation. Without any kind of randomization, differences in tumor baseline may be introduced that could alter the final study outcome. In most of the included studies it was not mentioned that blinding or randomization was performed, which may have introduced bias (Bebarta et al. 2003). Moreover, to ensure enough power of an experimental design, power calculations are an essential tool. None of the included studies described any kind of power calculation that may suggest lack of power in the included studies. These possible overestimations by studies included with bias may implicate that our observed effects may be less reliable. However, it may also be true that studies were correctly performed, but that experiments were only poorly reported, which is known from previous systematic reviews on animal studies that most studies poorly describe their *in vivo* experiments (Hooijmans et al. 2012). Therefore we would like to encourage to improve reporting of animals studies by using for instance the golden standard publication checklist (Hooijmans et al. 2011) or the ARRIVE guidelines (Kilkenny et al. 2010). Finally, a funnel scatter plot analysis suggests publication bias, which could have introduced an overestimation of our results as well.

A major remark regarding our results is that we did not look at side effects as outcome measure. This aspect may change the impact of our results. For instance, IP treatment in patients results in increased survival, but these patients experience more severe side effects (*e.g.* pain, fatigue and gastrointestinal

595 effects (Armstrong et al. 2006; Barlin et al. 2012; Jaaback et al. 2011). If the application of
596 chemotherapeutics in DDS would decrease side effects in IP treatment, this may be a major improvement
597 in patient quality of life. This also applies for active targeting or the specific DDS. Our results do not
598 suggest improved treatment by active targeting, however, if side effects would be decreased using active
599 targeting, active targeting may have a great advantage over passive targeting regardless of the lack of
600 enhanced efficacy. Moreover, results suggest that there is not a specific class of DDS that outperforms in
601 tumor growth inhibition or survival. Again, if a specific class of DDS would show considerably less side
602 effects, this class would be clinically very attractive although it does not outperform other DDS regarding
603 tumor size or survival in animal studies. The same is valid for the choice of cytostatic drug. Our results do
604 not suggest a specific higher efficacy for cisplatin, doxorubicin or paclitaxel if entrapped in a DDS
605 regarding survival in animal studies. However, if entrapment of one of these drugs results in significant
606 less side effects, this may be again of clinical importance and a major argument to entrap this specific
607 chemotherapeutic in a DDS, despite similar efficacies compared to the other drugs as found in this
608 systematic review. Although not in ovarian cancer, O'brien showed that free doxorubicin and pegylated
609 doxorubicin in treatment of metastatic breast cancer showed comparable overall survival with
610 significantly less cardiotoxicity in the pegylated liposomal doxorubicin group (O'Brien et al. 2004). As
611 only a few studies included in this systematic review addressed side-effects, an additional new systematic
612 review on animal studies with meta-analysis should be performed to assess the specific research question;
613 the effect of entrapment of chemotherapeutics in DDS on side effects.
614
615 In conclusion, delivery of chemotherapeutics with a DDS seems to be effective with regard to both tumor
616 size and survival in animal models. Results of this study support the claim that delivery of
617 chemotherapeutics is more effective compared to treatment with free chemotherapeutics, and that this
618 efficacy is not dependent on specific characteristics of DDS. Future well-designed *in vivo* studies
619 evaluating the efficacy of different characteristics of DDS on tumor size inhibition, survival and side
620 effects should be performed to identify important characteristics of DDS for clinical translation.

621

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811 TV, and Lengyel E. 2013. Urokinase plasminogen activator system-targeted delivery of nanobins
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813

814

Figure 1(on next page)

Figure 1

Figure 1. Flow chart of study inclusion . PubMed and EMBASE via OvidSP were searched using developed search strings to identify studies that used chemotherapeutics in a DDS in ovarian cancer animal models. All studies were first screened by title and abstract according to predefined inclusion and exclusion criteria. Subsequently studies were more specifically assessed by full text. Screenings were performed by two reviewers (RR and WD). Full text studies excluded for “others” were: 1) no full text was available or only an abstract that did not include sufficient information (n=12); 2) conference abstract of a previously assessed full-text study (n=5); 3) the study included only a biodistribution experiment (n=4).

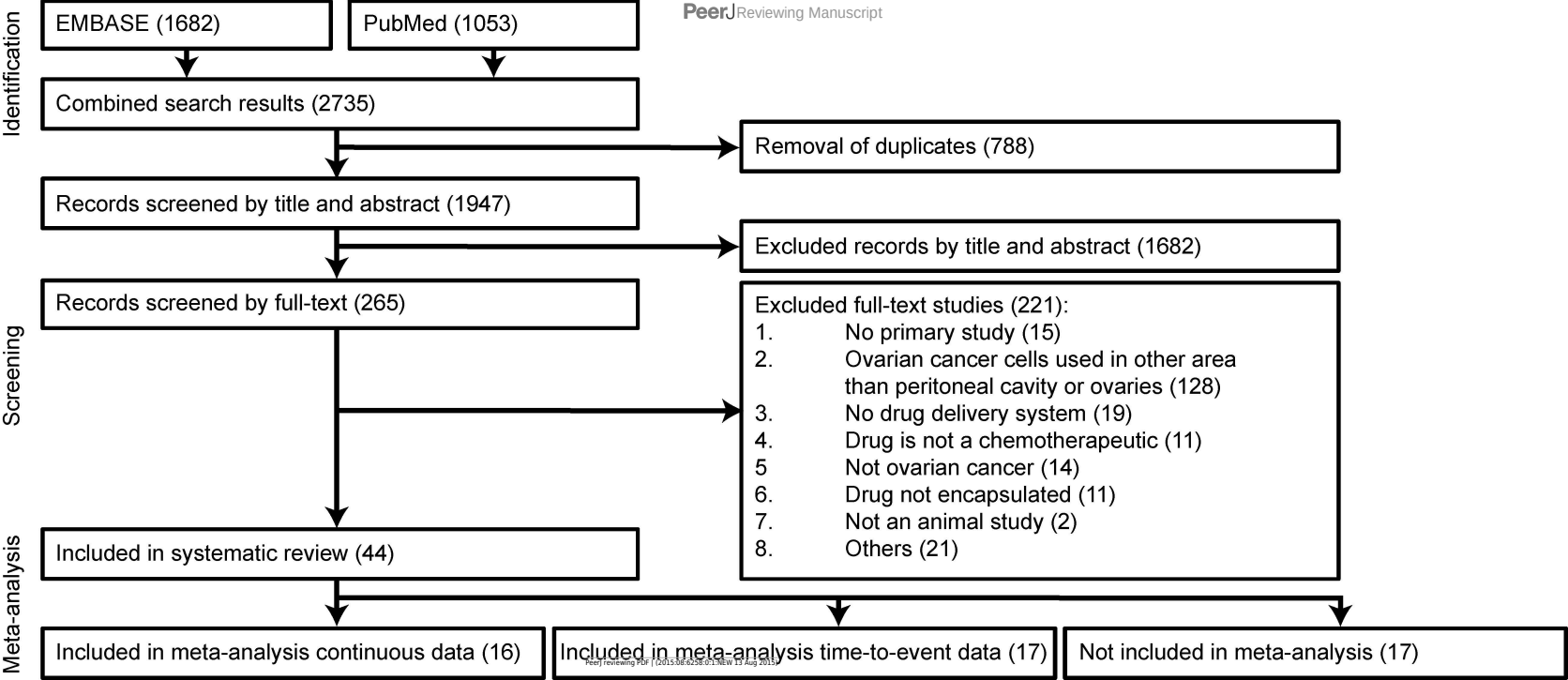

Identification
Screening
Meta-analysis
EMBASE (1682)
PubMed (1053)
Combined search results (2735)
Removal of duplicates (788)
Records screened by title and abstract (1947)
Excluded records by title and abstract (1682)
Records screened by full-text (265)
Excluded full-text studies (221):
1. No primary study (15)
2. Ovarian cancer cells used in other area than peritoneal cavity or ovaries (128)
3. No drug delivery system (19)
4. Drug is not a chemotherapeutic (11)
5 Not ovarian cancer (14)
6. Drug not encapsulated (11)
7. Not an animal study (2)
8. Others (21)
Included in systematic review (44)
Included in meta-analysis continuous data (16)
Included in meta-analysis time-to-event data (17)
Not included in meta-analysis (17)

Figure 2(on next page)

Figure 2

Figure 2. Risk of bias analysis . The risk of bias for all included studies was analyzed using several signaling questions. Depicted results are the answers for all studies per question.

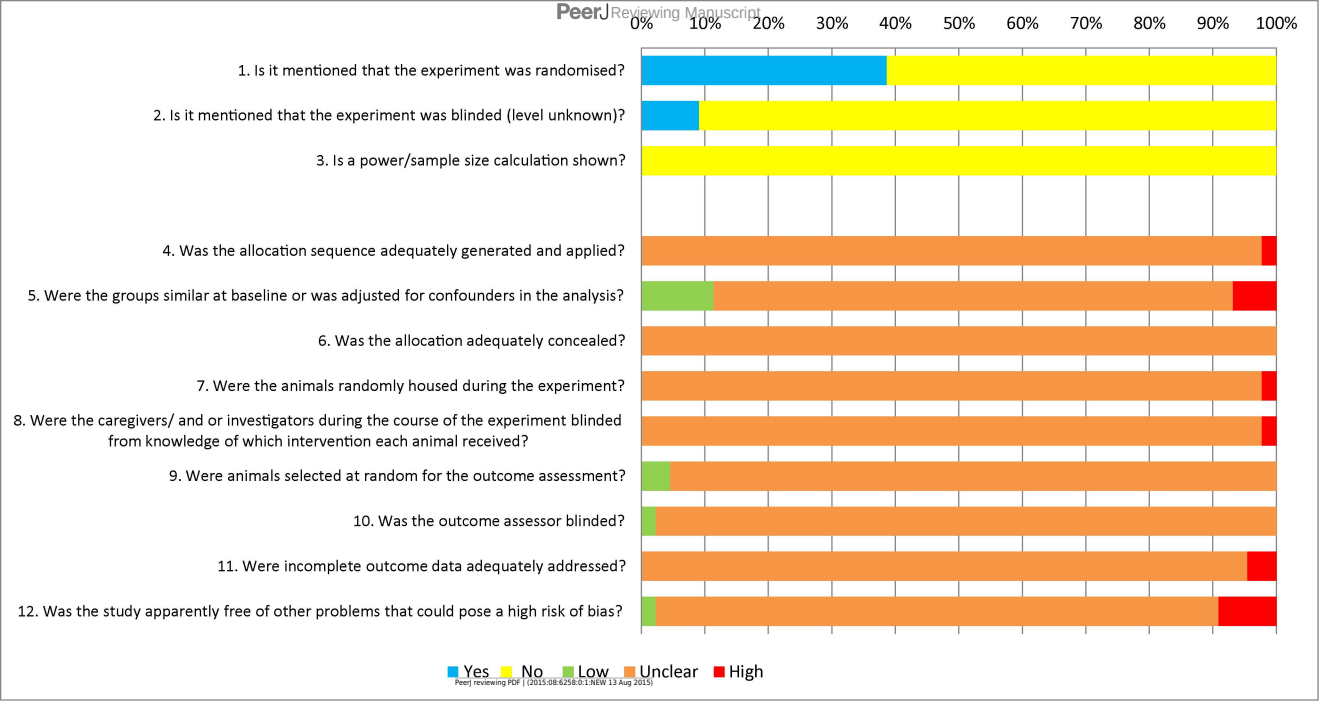

0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
1. Is it mentioned that the experiment was randomised?
2. Is it mentioned that the experiment was blinded (level unknown)?
3. Is a power/sample size calculation shown?
4. Was the allocation sequence adequately generated and applied?
5. Were the groups similar at baseline or was adjusted for confounders in the analysis?
6. Was the allocation adequately concealed?
7. Were the animals randomly housed during the experiment?
8. Were the caregivers/ and or investigators during the course of the experiment blinded from knowledge of which intervention each animal received?
9. Were animals selected at random for the outcome assessment?
10. Was the outcome assessor blinded?
11. Were incomplete outcome data adequately addressed?
12. Was the study apparently free of other problems that could pose a high risk of bias?
Yes
No
Low
Unclear
High

Figure 3(on next page)

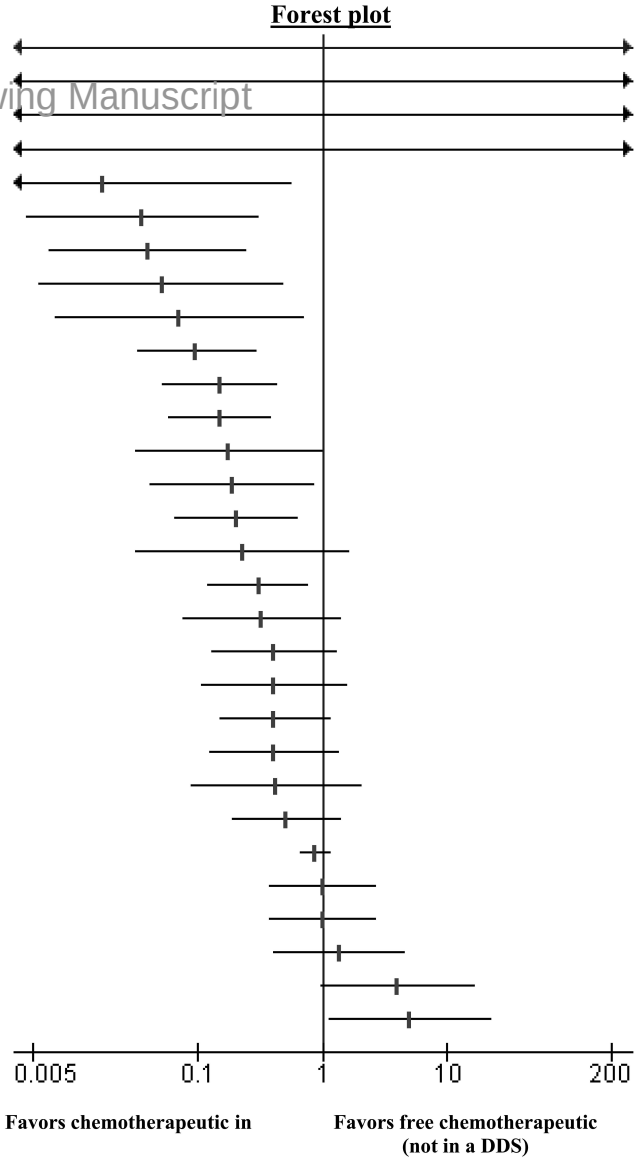
Figure 3

Figure 3. Effects of survival outcome measure of chemotherapeutics in a DDS compared to free chemotherapeutics (not in a DDS. A) The forest plot depicts hazard ratios with 95% confidence interval (CI) and the weight of the study. A hazard ratio below 1 indicates a smaller chance for the animals to die over the course of the experiment due to treatment with chemotherapeutics in a DDS. A hazard ratio higher than 1 suggests that animals have a smaller chance of dying when treated with the free chemotherapeutic control condition. Statistical significance was reached when hazard ratios with their 95% confidence interval did not include the value of 1. Numbers in brackets behind study names refer to details of the specific experiments; see supplementary material for details. B) Subgroup analysis for type of DDS, type of chemotherapeutic, targeted vs. non-targeted, IP vs. IV route of administration and inoculated cell type were performed. n is the number of experiments in the subgroups. I2 was used as a measure of heterogeneity.

A.

Overall hazard ratio

Study name	Hazard ratio [95% CI]
Chaudhury et al 2012 (1)	0.00 [0.00, 6.219E147]
Yang et al 2014 (2)	0.00 [0.00, 3.860E200]
Cirstoiu-Hapca et al 2010 (3)	0.00 [0.00, 3.244E28]
Cirstoiu-Hapca et al 2010 (4)	0.00 [0.00, 3.355E29]
Konishi et al 2012 (5)	0.02 [0.00, 0.56]
Pu et al 2014 (6)	0.04 [0.00, 0.31]
Winer et al 2010* (7)	0.04 [0.01, 0.24]
Cirstoiu-Hapca et al 2010 (8)	0.05 [0.01, 0.49]
Shaikh et al 2013 (9)	0.07 [0.01, 0.71]
Pastorino et al 2008 (10)	0.10 [0.03, 0.29]
Pastorino et al 2008 (11)	0.15 [0.05, 0.42]
Patankar et al 2013 (12)	0.15 [0.06, 0.39]
Pu et al 2014 (13)	0.18 [0.03, 0.98]
Winer et al 2010* (14)	0.19 [0.04, 0.86]
Tong et al 2014 (15)	0.21 [0.07, 0.63]
Cirstoiu-Hapca et al 2010 (16)	0.23 [0.03, 1.58]
Tang et al 2012 (17)	0.31 [0.12, 0.76]
Winer et al 2010* (18)	0.33 [0.08, 1.38]
Lee et al 2013* (19)	0.41 [0.13, 1.26]
Xiao et al 2009 (20)	0.41 [0.11, 1.54]
Tong et al 2014 (21)	0.41 [0.15, 1.12]
Li and Howell 2010* (22)	0.41 [0.13, 1.33]
Rapoport et al 2004 (23)	0.43 [0.09, 1.98]
Lu et al 2008 (24)	0.51 [0.19, 1.37]
Tong et al 2014 (25)	0.87 [0.67, 1.14]
Javid et al 2014 (26)	1.00 [0.39, 2.60]
Javid et al 2014 (27)	1.00 [0.38, 2.62]
Chaudhury et al 2012 (28)	1.36 [0.41, 4.52]
Pu et al 2014 (29)	3.95 [0.98, 15.93]
Pu et al 2014 (30)	4.98 [1.15, 21.54]



B.

Subgroups hazard ratio

Subgroup	Hazard ratio [95% CI]	n	I^2 (%)
Overall	0.39 [0.27, 0.56]	34	64
Type of DDS			
Liposomes	0.33 [0.17, 0.63]	12	77
Micro/nano-particles	0.38 [0.17, 0.83]	15	68
Type of chemotherapeutic			
Cisplatin	0.36 [0.18, 0.70]	7	51
Doxorubicin	0.28 [0.09, 0.87]	4	75
Paclitaxel	0.56 [0.38, 0.84]	20	53
Targeted vs. Non-targeted			
Targeted	0.24 [0.10, 0.57]	12	79
Non-targeted	0.48 [0.33, 0.69]	22	46
IV vs. IP administration			
IV	0.21 [0.08, 0.50]	9	84
IP	0.55 [0.36, 0.84]	19	52
Combination IP/IV	0.24 [0.11, 0.51]	6	0
Type of cell line			
SKOV-3	0.41 [0.23, 0.73]	9	49
OVCAR-3	0.46 [0.27, 0.79]	9	55
A2780	0.54 [0.18, 1.61]	7	77
ID-8	0.15 [0.05, 0.48]	3	39
IGROV-1	0.39 [0.03, 4.89]	3	60

Figure 4(on next page)

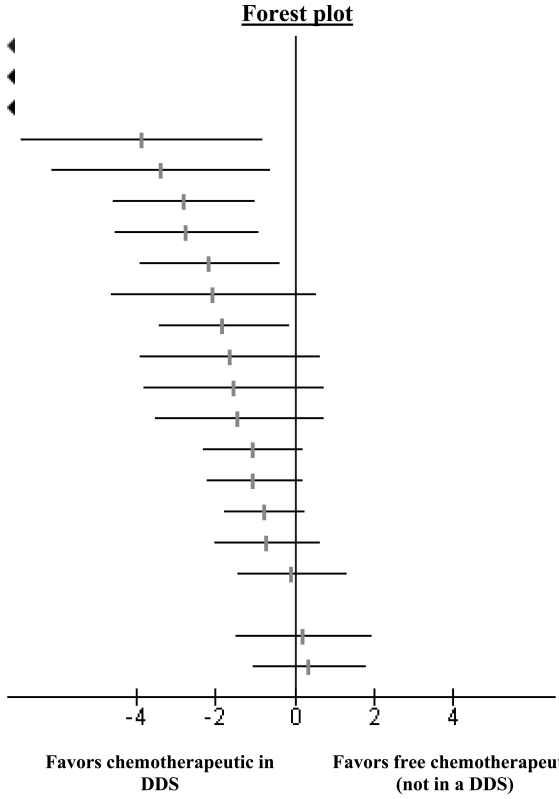
Figure 4

Figure 4. Effects on tumor growth inhibition outcome measure of chemotherapeutics in a DDS compared to free chemotherapeutics (not in a DDS). A) The forest plot depicts SMDs with 95% confidence interval (CI) and the weight of the study. A statistically significant difference between interventional conditions (chemotherapeutic in DDS) and control conditions (chemotherapeutics not in a DDS) was reached when the SMD with its 95% confidence interval was greater or smaller than zero. If below zero, the interventional condition is more efficient in reducing the tumor size, while if greater than zero, the control condition is more efficient in reducing the tumor size. Numbers in brackets behind study names refer to details of the specific experiments; see supplementary material for details. B) Subgroup analysis for type of DDS, type of chemotherapeutic, targeted vs. non-targeted and IP vs. IV route of administration were performed. n is the number of experiments in the subgroups. I2 was used as a measure of heterogeneity.

A.

Overall tumor growth inhibition

Study name	SMD [95% CI]
Javid et al 2014 (1)	-26.04 [-34.14, -17.95]
Javid et al 2014 (2)	-19.37 [-25.42, -13.33]
Lu et al 2007 (3)	-14.45 [-18.43, -10.46]
Ateh et al 2011 (4)	-3.91 [-6.95, -0.86]
Ateh et al 2011 (5)	-3.42 [-6.18, -0.67]
Patankar et al 2013-1* (6)	-2.82 [-4.60, -1.04]
Zhang et al 2013 (7)	-2.75 [-4.57, -0.94]
Yang et al 2014 (8)	-2.18 [-3.92, -0.43]
Cirstoiu-Hapca et al 2010 (9)	-2.07 [-4.63, 0.49]
Zhang et al 2013 (10)	-1.82 [-3.45, -0.19]
Sengupta et al 2012 (11)	-1.67 [-3.94, 0.60]
Xu et al 2006 (12)	-1.54 [-3.80, 0.72]
Paraskar et al 2010 (13)	-1.43 [-3.55, 0.68]
Ye et al 2013 (14)	-1.07 [-2.32, 0.18]
Li and Howell 2010* (15)	-1.04 [-2.24, 0.15]
Gilmore et al 2012 (16)	-0.77 [-1.76, 0.22]
Xiao et al 2009 (17)	-0.71 [-2.01, 0.60]
Jin et al 2007 (18)	-0.07 [-1.46, 1.31]
Konishi et al 2012 (19)	Not estimable
Xu et al 2006 (20)	0.22 [-1.49, 1.93]
Jin et al 2007 (21)	0.36 [-1.05, 1.77]



B.

Subgroups tumor growth inhibition

Subgroup	SMD [95% CI]	n	I^2 (%)
Overall	-2.70 [-3.81, -1.59]	21	86
Type of DDS			
Micelles	-0.57 [-1.51, 0.37]	3	0
Liposomes	-1.82 [-3.52, -0.13]	3	60
Micro/nano-particles	-4.44 [-6.24, -2.65]	13	90
Type of chemotherapeutic			
Cisplatin	-0.54 [-1.15, 0.07]	7	0
Paclitaxel	-4.15 [-6.20, -2.09]	9	90
Targeted vs. Non-targeted			
Targeted	-2.05 [-3.25, -0.86]	4	33
Non-targeted	-2.95 [-4.31, -1.59]	17	89
IV vs. IP administration			
IV	-2.09 [-3.26, -0.92]	4	0
IP	-3.01 [-4.34, -1.67]	16	89

Figure 5(on next page)

Figure 5

Figure 5. Funnel scatter plot of time-to-event studies. Hazard ratios with a 95% confidence interval were extracted and used to create a funnel scatter plot using Review Manager. Bullets represent individual experiments from included studies. The x-axis shows the hazard ratio and the y-axis represents the standard error of the log(hazard ratio). The funnel plot is missing studies in the bottom right area in which studies with a negative outcome are expected. Since there are no studies in this area, publication bias is suggested.

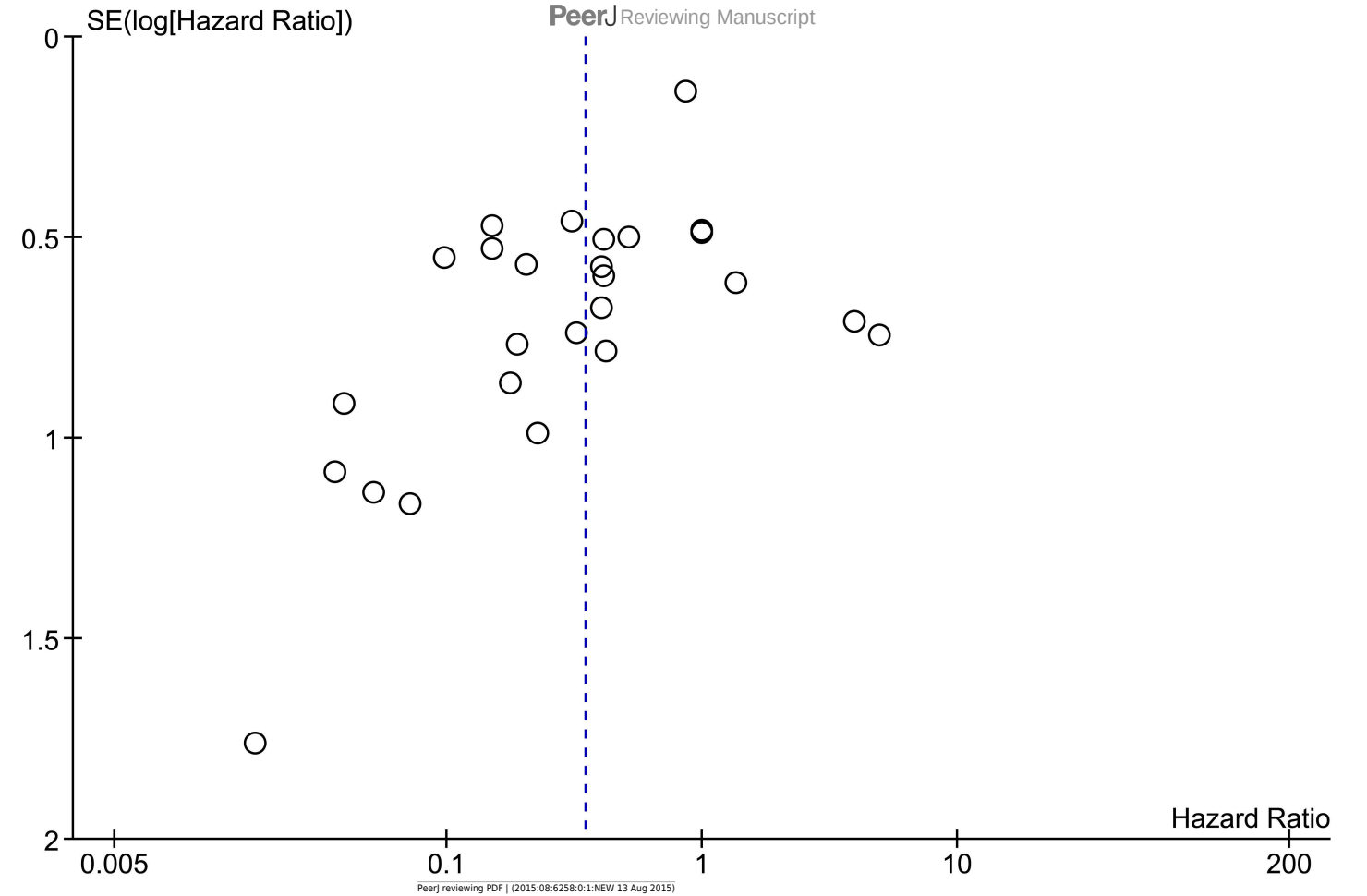


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Table 1

Table 1. Overview of study characteristics collected after inclusion in systematic review.

1 **Table 1. Overview of study characteristics collected after inclusion in systematic review.**

Publication details	Drug delivery system details	Animal details	Animal model details	Treatment details	Results details
Title	Delivery system name	Species	Cell type/line	Experimental groups	Drop-outs
Year	Material	Strain	Number of cells	# Animals per group	Tumor size evaluation method
Journal	Preparation method	Genotype	Inoculation area	Administration route	Outcome measures
Volume	Particle size	Sex		Dose	Side-effect measures
Issue	Zeta-potential	Age		Regime	
Pages	Cytostatic drug	Weight		Inoculation time	
	Drug concentration in particles			Follow-up time	
	Release characteristics				
	Active/passive targeting				
	Antibody/antigen				
	Surface modifications				
	Special characteristics				

2

3